Journal of DAIRY SCIENCE

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JOURNALS NEEDED

The May, June, and September, 1958, issues of this Journal are needed for orders, subscriptions, and late memberships. Send them to the Garrard Press, 510 North Hickory Street, Champaign, Illinois. The Association will pay $0.50 plus postage for each copy.

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June 18 to 20, 1959

JOURNAL OF DAIRY SCIENCE

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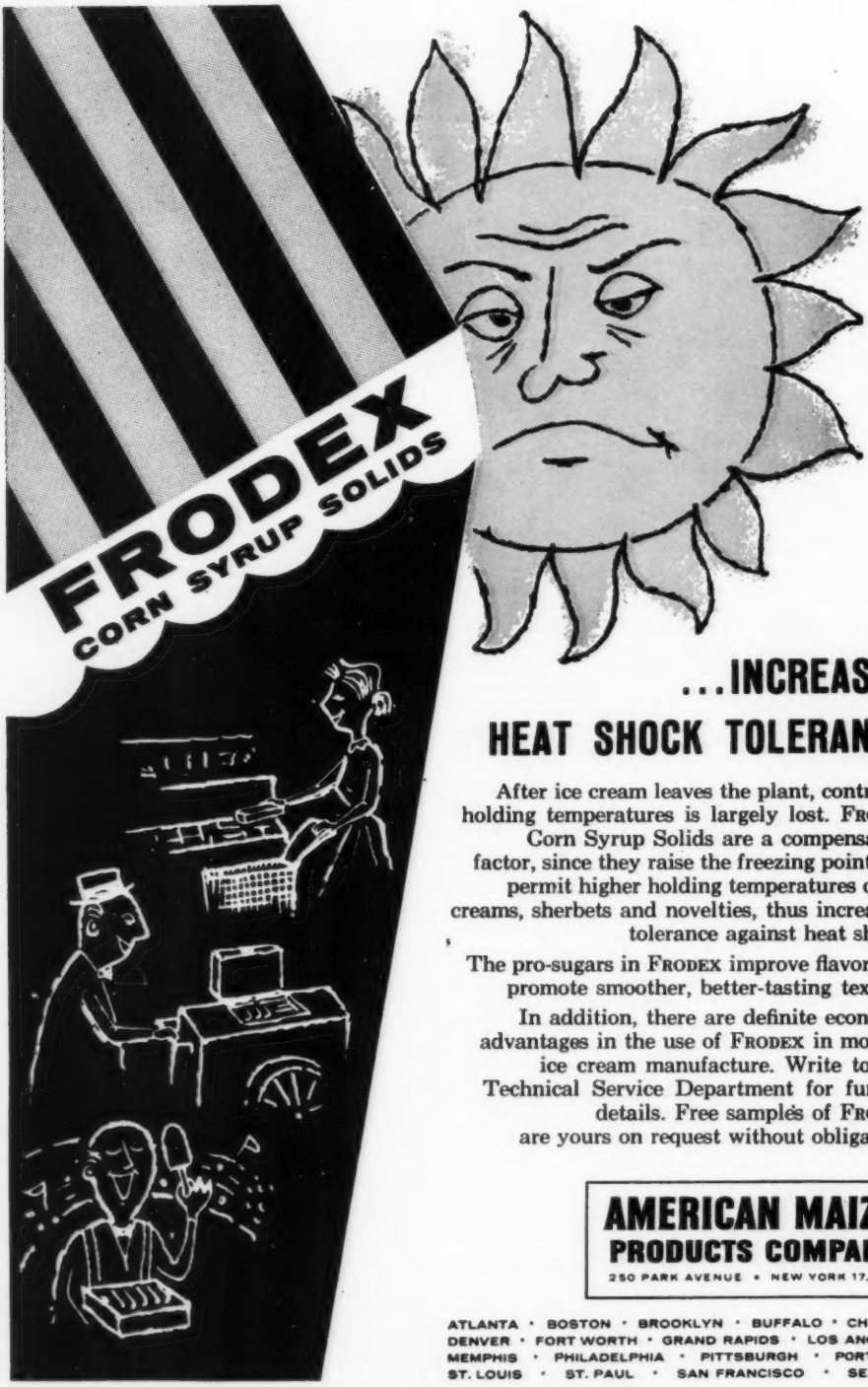

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PEOPLE AND EVENTS

Bendixen Honored by Washington State

The program of the 28th Annual State College of Washington Institute of Dairying took a surprise turn at the banquet sponsored by the student branch of the American Dairy Science Association on March 10. The surprise was on H. A. BENDIXEN, Professor of Dairy Science and Director of the institute.

Dr. and Mrs. Bendixen

Dr. Bendixen found himself the surprise featured guest, as 200 former students, friends, and associates turned up for the dinner to honor him for 32 years of service to Washington State College and to the Washington dairy industry. Another 200 well-wishers sent greetings in the form of personal letters.

Laurels presented to the veteran educator included $1,000 as an appreciation gift, a leather-bound volume of personal letters, a plaque, a humorous doctoral degree, and a cane with 32 service bars.

Climax of the evening was the presentation of a certified check for $1,000 contributed by former students, representatives of the dairy industry, associates, and friends.

A plaque from Washington's dairy industry was presented by A. F. BIRD, Seattle, secretary of the Washington State Dairy Foundation. The plaque was inscribed with a tribute to Dr. Bendixen as teacher, counselor, and friend.

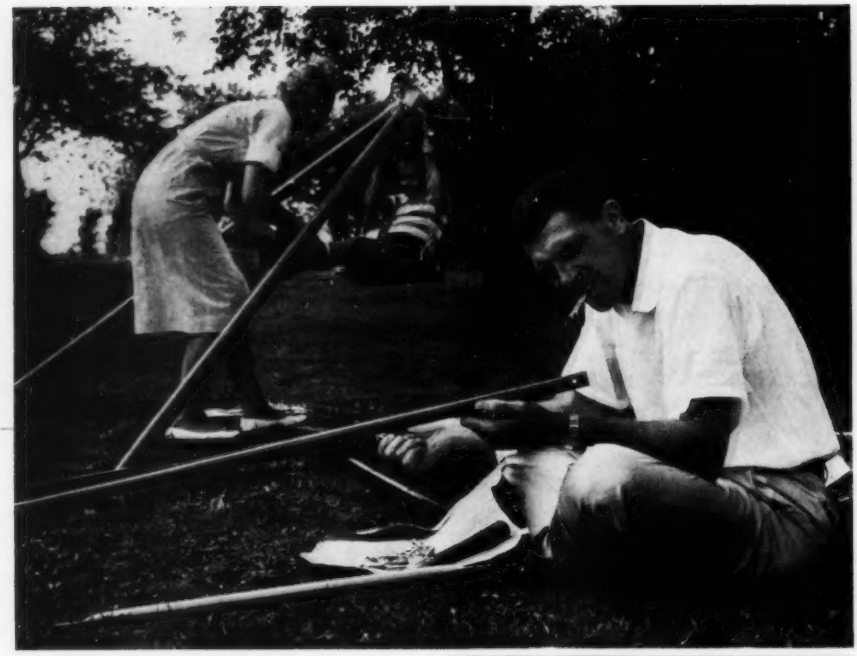
Left to right: Walter R. Ullrich, President of the Washington State Dairy Foundation; A. Frank Bird, Secretary of the Washington State Dairy Foundation, presenting plaque of appreciation to Dr. H. A. Bendixen.

A leather-bound volume of personal letters was presented by L. J. MANUS, Washington State College dairy scientist. The letters from WSC dairy graduates, associates, and friends came from 28 states, Germany, England, Panama, Korea, and the Philippines.

The cane—which was made by RUPERT SEALS, Lexington, Ky., a graduate student, was a gift from the Dairy Science graduate students at WSC.

The State College of Washington staged its 28th annual information-packed Institute of Dairying March 9-12 with numerous outstanding out-of-state guest speakers, including W. V. PRICE of the University of Wisconsin, A. W. FARRALL, Head, Dept. of Agricultural Engineering, Michigan State University, W. L. PATTERSON, Chief, U. S. Dairy Products Laboratory, Washington, D. C., D. H. JACOBSEN, Research Director, American Dairy Association, Chicago, Ill., S. KENT CHRISTENSEN, Department of Agricultural Economics, Oregon State College, Corvallis, Oregon, J. L. BARNHART, Department of Dairy Husbandry, University of Idaho, Moscow, Idaho, HAROLD WAINESS, Consultant in Sanitary Science and Public Health, Chicago, Illinois, GEORGE L. ANDREWS, T. E. A., Inc., Flushing, N.Y., T. LEWANDOWSKI, Technical Director, Bonewitz Chemicals Co., Burlington, Iowa, and D. C. STAHLE, Product Manager, Crown Zellerbach Corporation, San Francisco, Calif.

At the special session for milk sanitarians sponsored by the Washington Milk Sanitarians Association the main emphasis was given to a discussion of efficiency in milking parlor operations. Lead-off speakers were M. L. STROMMER, State Department of Agriculture, Olympia,



BUILDING

A GUIDE TO GOOD EATING — USE DAILY

DAIRY FOODS

3 to 4 glasses milk—children • 4 or more glasses—teenagers • 2 or more glasses—adults • Cheese, ice cream and other milk-made foods can supply part of the milk

MEAT GROUP

2 or more servings • Meats, fish, poultry, eggs, or cheese—with dry beans, peas, nuts as alternates

VEGETABLES AND FRUITS

4 or more servings • Include dark green or yellow vegetables; citrus fruit or tomatoes

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4 or more servings • Enriched or whole-grain added milk improves nutritional values

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The efficiency with which food proteins are used depends upon the balance of essential amino acids which are simultaneously supplied with sufficient calories from fat and carbohydrate . . . and with needed minerals and vitamins. Not all food proteins, individually consumed, are equally well utilized for building body tissues . . . because food proteins vary in structure . . . in their amino acid content. However, food proteins are seldom eaten individually. Instead, proteins of different foods are combined in meals . . . and the amino acids in each tend to supplement each other . . . as do the other nutrients present.

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¼ from milk, cheese and ice cream . . . ⅓ from the meat group . . . ¼ from vegetables and fruits . . . 1/7 from breads and cereals.

When combined in well-prepared meals, foods selected from each of these four food groups can provide adequate protein . . . while satisfying the tastes, appetites and all other nutrient needs of all members of the family . . . young and old.

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DR. C. H. ZUROSKE of the Department of Agricultural Economics, at the State College in Pullman, and H. V. COPENHAVER of the Arden Farms Co., Moscow, Idaho. Regular committee reports were heard. Professor C. C. PROUTY of the State College and president of the Washington Milk Sanitarians Association conducted the business sessions. Officers elected for the ensuing year are: President—H. W. JANZEN, State Department of Agriculture, Yakima, President-elect — LOUIS ARRIGONI, Consolidated Dairy Products Co., Seattle and Secretary-Treasurer—F. W. LOGAN, Seattle-King County Health Department, Seattle, Washington.

Recent Deaths

WILLES BARNES COMBS, 66, professor of dairy industry at the University of Minnesota, died March 7, 1959, in St. Paul following a three-month illness.

A staff member at the university since 1924, Combs was noted for his research work in cheese, ice cream, and butter production. He was instrumental in the development of Minnesota blue cheese and Nuworld cheese.

Combs was an early promoter of the dairy manufacturing industry and research in Minnesota and was secretary of the Minnesota Dairy Industry committee for some 20 years.

Born in Memphis, Mo., he received his B.S. in 1915 and M.S. in 1917 from the University of Missouri. He then taught at Rutgers University, the University of Missouri, and Penn State University before coming to Minnesota.

He was author of some 75 publications, including a book "Milk and Milk Products." He was a Fellow in the American Association for the Advancement of Science and was a member of A.D.S.A., the American Chemical Society, Sigma Xi, Alpha Zeta, and Gamma Sigma Delta.

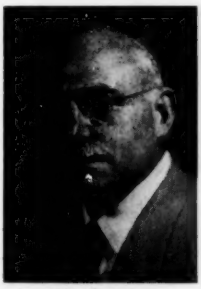
In his memory, the library in the new Dairy Industry building now being completed on the St. Paul campus will be named the Willes Barnes Combs Room. Memorials may be sent to the Willes Barnes Combs Memorial Library fund, Dairy Dept., University of Minnesota.

Survivors include his wife, Nancy; two daughters, Mrs. Fred Corbett, Venice, Fla., and Mrs. Leo Weber, Reno, Nev.; a sister, Mrs. Alice Carman, Maplewood, Mo.; and a brother, C. Barnes Combs, Gravois Mills, Mo.

A. ERLE BANKS, 63, president of the Associated Dairy Products Co., Glendale, Ariz., died June 13, 1958. Mr. Banks served the dairy industry in Arizona from 1910 until his death. He became president of Associated in 1939. His company merged with Beatrice Foods Co. in 1956.

Mr. Banks is survived by his wife, LENNIE; a daughter, ELEANOR; and a son, J. A. BANKS.

NANDOR PORGES, 57, a microbiologist with the USDA since 1937, died suddenly April 1, 1959, at the home of his mother in New Brunswick, N. J. He had attended the funeral of his father the day before.



N. Porges

Dr. Porges is widely known for his work in developing a simple, inexpensive process for the disposal of dairy wastes. He was a member of the staff of the Agricultural Research Service's Eastern Utilization Research and Development Division in Wyndmoor, Pa.

A native of Hungary, he was a graduate of the University of Massachusetts, Amherst, and received his M.S. and Ph.D. degrees from Rutgers University, New Brunswick, N. J.

The Dairy Waste Unit, which Dr. Porges headed, was awarded the USDA's Superior Service Award. Other honors bestowed on him include the Chilean Nitrate of Soda Fellowships 1927-29, Sigma Xi honor for research in 1931, Phi Tau Sigma, the honor society of food science, 1956. He was a member of A.D.S.A., the American Chemical Society, the Society of American Bacteriologists, the Iowa Academy of Science, the American Association for the Advancement of Science, the Society for Industrial Microbiology, and the Federation of Sewage and Industrial Wastes Association. He authored more than 65 scientific papers on such subjects as waste disposal, citric acid, gluconic acid, rot resistance of fabrics, rubber from goldenrod leaves, utilization of peanut and sweetpotato wastes, and the production of vitamin B_{12} from sludge.

Survivors, in addition to his mother, include his wife RUTH; a son ARNOLD, a dental student at the University of Pennsylvania; a daughter, MRS. BARBARA COHEN; a grandson; four brothers, WALTER, FREDERICK, RALPH, and DAVID; and three sisters, MRS. JOSEPHINE STAHAN, MRS. MINA MEYER, and MRS. ROSALIE MARX. He was active in the Hebrew Sunday School Society, was a member of the Four Freedoms Lodge, B'nai Brith, a member of Rodolph Zadek Synagogue, Philadelphia, and an honorary mmeber of Ohav Emmert Synagogue, New Brunswick.

NATHANIAL NEWTON ALLEN, 57, professor of dairy husbandry at the University of Wisconsin, died suddenly March 7, 1959, at Madison, Wis.

Dr. Allen was a native of Missouri and graduated from the University of Missouri in 1929. He received his Ph.D. at the University of Minnesota in 1935 and was on the staff

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there until 1942. He served at the University of Vermont until 1945 when he joined the Wisconsin staff.

He was widely known for his ability to translate technical nutrition information in a way that could be understood by dairy farmers. He had a national farmer audience with a regular column on dairy, feeding, and management in "Hoard's Dairyman." At Minnesota he developed a standard test for fat analysis in blood while conducting basic research on factors influencing fat levels in blood and milk. His current research dealt with the importance of the physical status of the ration on rumen function and butterfat test of the milk.

Dr. Allen served as a director and vice president of the A.D.S.A. and was a member of the Advisory Committee of the National Feed Manufacturers Association. He was also a member of the Madison First Baptist Church, a Missouri Masonic Lodge; and Acacia, Alpha Zeta, Gamma Alpha, and Sigma Xi fraternities.

Survivors include his wife; three daughters, Mrs. Joseph Cattellino, Saxon, Wis., and Virginia and Sarah, both at home; three sons, James, Aberdeen, S. D., Edward, Minneapolis, Minn., and David, at home.

Frank W. Fitch, 64, extension dairyman of the Georgia Agricultural Extension Service, died December 20, 1958, after a brief illness. He retired from his job in July, 1957, after 37 years of service.

F. W. Fitch

Among his many accomplishments were his early work in artificial insemination of dairy cattle in Georgia; his training for the first livestock judging team, first dairy cattle judging team, and first dairy demonstration team to represent Georgia 4-H Clubs in national contests; and his help in the assembly of the foundation herd of Jerseys for the Georgia State Prison, the only herd ever assembled in which all the cows classified either very good or excellent. In 1948 he was selected by the Georgia Dairy Association as "Man of the Year in Dairying."

Mr. Fitch was born in Cornish, N. H., and graduated from the University of New Hampshire in 1917. He was a member of Epsilon Sigma Phi, Alpha Gamma Delta, the A.D.S.A., and other professional organizations. He was an honorary member of the local Student Branch of A.D.S.A., and was an officer in the First Presbyterian Church of Athens, Ga.

Survivors include a daughter, a son, three brothers, and six grandchildren.

Ralston-Purina Awards

Some 16 winners and alternates have been announced for the 1959 Ralston-Purina Research Fellowships. The awards committee made its selection in March.

Members of the selection committee are R. H. Thayer, Oklahoma State University, representing the Poultry Science Association; J. B. Frye, Louisiana State University, representing the American Dairy Science Association; Robert Mac Vicar, Oklahoma State University, representing the American Association of Land Grant Colleges and Universities; L. E. Hanson, University of Minnesota, representing the American Society of Animal Production; and C. A. Brandly, University of Illinois, representing the American Veterinary Medical Association.

The winners are as follows, giving name, institution of undergraduate, and institution of graduate, in that order:

Dairy Husbandry

Emmett Keith Inskeep, West Virginia University, University of Wisconsin
Curtis W. Richardson, Oklahoma State University, University of Missouri
Alan S. Todd, University of Georgia, University of Georgia

Poultry Husbandry

Bruce E. McDonald, University of Alberta, University of Alberta
Thomas F. Simco, University of Arkansas, University of Arkansas
Woodie P. Williams, Jr., Mississippi State University, Texas A&M

Animal Husbandry

Earl F. Ellington, University of Kentucky, University of California
Don Ovid Pinney, University of Illinois, Oklahoma State University
Margery Jean Twombly, University of California, University of California

Veterinary Medicine

Max James Freeman, Alabama Poly. Insti., University of Wisconsin

Alternates

Robert J. Bingham, Utah State University, University of Minnesota
William H. Feaster, Rutgers University, Virginia Poly. Insti.
Robert G. Rateliff, Mississippi State University, Texas A&M
Byron F. Miller, Kansas State College, Kansas State College
Everett Dean Schuman, Purdue University, University of Wisconsin
Paul J. Reynolds, Purdue University, Cornell University

South Dakota News

South Dakota's Depts. of Dairy Husbandry and Bacteriology will have a new building in the near future. The Governor has signed a bill which appropriates $800,000 for the construction of such a building to house classrooms, teaching and research laboratories, dairy processing facilities, and offices for these educational areas.

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(1) Standard Methods for the Examination of Dairy Products, ed. 10, New York, American Public Health Association, 1953. (2) Committee Report, Am. J. Pub. Health 42:1131 (Sept.) 1952. (3) Microbiological Methods, report at 66th Ann. Meet. Assn. Official Agricultural Chemists, Sept. 29, 1952: J. Assn. Official Agr. Chem. 36:91 (Feb.) 1953. (4) Standard Methods for Examination of Water, Sewage and Industrial Wastes, ed. 10, New York, American Public Health Association, 1955.

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Rusoff Honored at L.S.U.

A memorial award honoring one of Louisiana's most prominent figures in chemistry and chemical engineering was awarded for the second time to a Louisiana State University professor.

L. L. RUSOFF, dairy nutritionist and professor of dairying at LSU, was presented the Charles E. Coates Memorial Award at a dinner attended by members of the Baton Rouge chapters of the American Chemical Society and the American Institute of Chemical Engineers, who sponsor the annual award. The presentation was made by T. H. MIDDLETON, president of LSU.

L. L. Rusoff

The award honoring the late Dr. Coates was established last year. He was head of the LSU chemistry department for more than 40 years and Dean of the Audubon Sugar School. In 1934 the LSU chemistry laboratory was dedicated in his name as a tribute to his significant services.

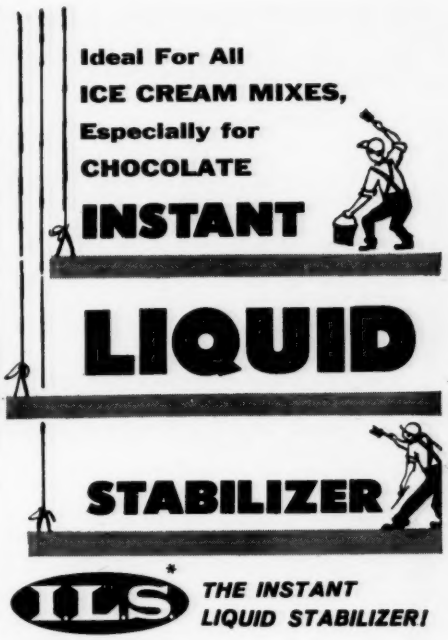
The award is presented to a leading chemist or chemical engineer in the Baton Rouge area. Selection is based on contributions to chemistry or chemical engineering, including publications, activity in ACS or the AICE, and for community activities.

Dr. Rusoff was selected for his pioneering work in nutritional antibiotic research and other studies in biological and nutritional chemistry which have led to the publication of more than 100 scientific papers. He developed a new method of preserving cheese from spoilage by using antibiotics, this discovery in turn leading to the current use of antibiotics in the preservation of poultry and fish.

As a member of the American Chemical Society, he has served on various committees, as chairman of Chemical Progress Week and as chairman of the local section. In the community he is a board member for the Citizens Committee for Education and is regional director of the Louisiana Science Fair for the fourth consecutive year.

A native of Newark, N. J., Dr. Rusoff attended the Oak Ridge Institute of Nuclear Studies in 1957 and is a member of the Governor's State Committee on Nuclear Energy. He received degrees from Rutgers University, Pennsylvania State University and finally his Ph.D. degree in agricultural biochemistry and nutrition at the University of Minnesota.

The LSU scientist has been active in civic and religious activities in Baton Rouge. He is a counselor for the LSU Hillel Foundation and



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Re: UHT Pasteurization

Dear XXXXXX:

In answer to your letter of the 30th, we, at Creamery Package, offer several approaches to UHT through our CP Crescent and Crescent SC plate heaters with pressurized water heating units; our CP Swept Surface heater; and our CP Steam Infuser (direct contact steam heater). In addition, CP Vac-Heat units are offered for UHT or as accessory flavor treating units to UHT pasteurizers.

Creamery Package offers a choice of UHT equipment that allows our competent branch engineers and technologists to aid a processor in the selection of equipment which will best fit his particular needs and assure him of maximum bacterial kill, optimum flavor and preservation of the natural protein stability.

Although CP can furnish equipment to heat to any temperature up to 300°F. and above, we suggest that fluid milk processors start near the lower temperature limits of the UHT range, for this will assure them of an extension of shelf life consistent with present methods of post-pasteurization handling and will yield excellent flavor. CP installations can be modified for higher temperatures without loss in original investment at a later time if basic research proves that higher temperatures are really beneficial.

Your comments and questions were most welcome.

Sincerely yours,

H. L. Mitten, Jr.

H. L. Mitten, Jr.
Assistant to Vice President

HLM: jkw

is a member of the University Religious Council. He is listed in "American Men of Science," "Who's Who," "Chemical Who's Who," and others.

He joined the LSU faculty in 1942.

Louisiana News

Fifty people from Louisiana and five neighboring states registered for the third annual Dairy Products Technology Short Course, March 11-12.

This short course was designed to meet the needs of production superintendents, laboratory technicians, fieldmen, quality control men, and all other interested persons.

Speakers on the program were from the field of dairy manufacturing, the LSU faculty and Southeastern Louisiana College.

A luncheon, sponsored by the local dairy products plants, was served on the second day at the Faculty Club.

So far, the attendance at these short courses has been good and the consensus is that the time has been well spent.

Nebraska News

P. A. DOWNS, professor of dairy husbandry at the University of Nebraska, will retire in June after 36 years of service to the University. In honor of his long service, he and Mrs. Downs were honored at a recognition dinner May 13.

Dr. Downs started coaching the dairy products judging team in 1924 and a Nebraska team has been at each national contest since that date. A member of the American Dairy Science Judging Contest Committee for many years, he is well-recognized for his ability in this area of work.

He has been a member of A.D.S.A. since 1926, serving on the Board of Directors (1944-47), as chairman of the manufacturing section (1939), and presently as a member of the committees on dairy products judging contest and butter.

Dr. Downs was largely responsible for organizing the Nebraska Butter Institute and has been its secretary since it began in 1941.

Rhode Island News

W. M. ETGEN has joined the faculty of the University of Rhode Island Animal and Dairy Husbandry Dept. He replaces G. K. WILDES who resigned to take a position with the Maine Extension Service.

Dr. Etgen received his B.S., M.S., and Ph.D. degrees from Ohio State University. During the past four years he has been on the staff at Ohio State. Before 1954 he served as herdsman and later manager of Hoard's Dairy Farm, Ft. Atkinson, Wis. At Rhode Island he will be in charge of the dairy farm operations and will handle courses in dairy management.

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the Charles H. Hood Foundation were awarded to upper-classmen with high scholastic standing and an interest in dairying. The awards went to DONALD GILBERT, a senior, and JAMES WHEATON III, a Junior.

CLAUDE DESJARDINS, a junior in the College of Agriculutre, was the recipient of a $300 scholarship sponsored by the Charles M. Cox Feed Co.

Scientists Find New Milk Protein

Clue as to why evaporated and condensed milks often gel during storage may lie in a newly discovered milk protein, it was reported recently by the Eastern Utilization and Development Division of the U. S. Department of Agriculture.

The new protein, a constituent of alpha casein, differs in phosphorus content. It is called alpha-casein and, unlike most other milk proteins studied, cannot be precipitated with calcium salt. This fact, scientists believe, may lead to a solution of the gelling problem and also be important in stabilizing milk's calcium-casein complex.

Breeding Better Dairy Cattle for the South

The Southern Regional Committee on Breeding Better Dairy Cattle for the South held its two-day conference April 6-7 at the Louisiana State University. Cattle researchers from seven states attended the meeting.

Delegates in attendance represented colleges, universities, and the U. S. Department of Agriculture. They discussed mutual problems and progress in the improvement of dairy cattle.

The first day's program included discussions of the regional annual report and methods of evaluating genetic progress. There were also final reports on standards of eligibility of records to be used in analysis of experimental data and inter-regional udder palpation analyses. Dr. Frye, Head, L.S.U. Dairy Department, reported on the Louisiana breeding project.

During the morning of the second day the agenda included diseases and parasites of dairy cattle in relation to adaptability; new breeding concepts presented at International Genetics Congress; report on Fourth Inter-American Livestock Production Meeting, Jamaica, July, 1958; and a workshop discussion of concepts of future physiological research in relation to adaptability.

At the afternoon session plans were made for next year's meeting, to be in April at the Texas A. & M. College.

Ohio State News

About 450 persons attended the 26th Annual Dairy Technology Conference at Ohio State

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University, Feb. 3-5. The programs consisted of five main sections: "Field Program," "Dairy Engineering and Sanitation," "Plant Management and Operations," "Quality Control and Cultured Dairy Products," and "Ice Cream Operations."

One of the highlights of the conference was the banquet sponsored by the Dept. of Dairy Technology and the four Ohio Dairy Technology Societies—Maumee Valley, Northwestern Ohio, Central Ohio, and Cincinnati. The speaker was HARRY BOWSER, sales development manager, Dairypak-Butler, Inc., Cleveland. His topic was "Working with People."

The sixth Annual Dairy Technology Career day was held on April 11.

H. E. KENNEDY has been appointed a research associate in the Dept. of Dairy Technology. Dr. Kennedy will be conducting research on antibiotics in milk on a project supported by the U. S. Public Health Service.

Dr. Kennedy, a graduate from Atlantic Christian College in North Carolina, received his M.S. and Ph.D. degrees from North Carolina State College, Raleigh. Since 1954, he has been working with the North Carolina Sanatorium System as a research scientist. His post doctorate work has been concerned with the survival of cultures of tubercle bacilli under various conditions of growth.

T. V. ARMSTRONG, assistant professor in dairy technology since 1937, has retired and has been appointed Assistant Professor Emeritus by the O.S.U. Board of Trustees.

He received his B.S. degree in 1921 from Ohio State and conducted special studies for an additional year before becoming quality supervisor for Sealtest, Canton, O. After 12 years with Sealtest, he returned to the campus and during the past 22 years has been in charge of the department's audit program and Ohio examinations of weighers, samplers, and testers. He has also assisted in teaching and has been active in research on analytical methods for milk and milk products.

H. E. RANDOLPH, M.S., Ohio State 1958, has been appointed as Assistant and Analyst in the Department of Dairy Technology.

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T. D. HARMAN, Assistant Professor in the Department of Dairy Technology, has resigned to become a Bank Executive in his home town of Circleville, Ohio.

An Industry-wide Steering Committee has been organized to administer the Dairy Products Project of the Ohio State Fair. The members include, EARL POLING, and KEITH BERG, Ohio Division, the American Dairy Association; FRED GREINER, the Ohio Dairy Products Association; HERBERT JOHNSON, Ohio Milk Producers Federation; EMERSON MIZER, Ohio State Fair Board; I. A. GOULD, The Ohio State University; and ROWLAND BISHOP, State Fair Board Manager, ex-officio.

Plans are being made to continue the operation of the Dairy Products Building as in the past.

About 30 Sanitarians and 25 Industry representatives attended the 8th Annual Ohio Milk Sanitation and Public Health Short Course, at the Ohio State University, March 16-20.

W. M. ROBERTS, Professor of Dairy Manufacturing, North Carolina State College, Raleigh, N. C., delivered the opening address, "Regulations—Aids or Hindrance to the Dairy Industry." Other visiting speakers included, R. B. DOUGLAS, Beatrice Foods Co., New Bremen, O.; J. D. BOWERS, The Borden Co., Columbus, O.; GLEN WAGNER, Northwestern Cooperative Sales Ass'n., Toledo, O.; J. H. ERB, The Borden Company, Columbus, O.; DALE E. SEIBERLING, Klenzade Products, Inc., Beloit, Wis.; F. HOLMAN, Surge Co., Chicago, Ill.; L. C. PECKHAM, U.S.P.H.S., Chicago, Ill., L. C. BURKEY, Creamery Package Co., Toledo, O.; K. T. YODER, Diamond Milk Co., Columbus, O.; E. P. HICKS, Taylor Instrument Co., Columbus, O.; R. STAFFORD, Diamond Milk Co., Columbus, O.; J. O. ROBERTS, R&D Laboratory, Columbus, O.; C. K. ORTMAN, M&R Dietetics Labs., Inc., Columbus, O.; T. A. BATSCHE, City Health Department, Cincinnait, O.

The Short Course was sponsored cooperatively by the Ohio Dept. of Health, the Ohio Dept. of Agriculture and the Ohio State University.

Kansas News

The Kansas Ice Cream and Milk Institute and the Kansas Butter and Cheese Institute were merged on Feb. 5, 1959, at Wichita.

Speakers on the program included C. L. NORTON, head of the Dept. of Dairy Husbandry, Kansas State University; GEORGE NEELEY, National Association of Sanitary Milk Bottle Closure Manufacturers of Philadelphia; REX PAXTON, director of public relations, Sutherland Paper Co., Kalamazoo, Mich.; BRUCE ROWLEY, Kansas Dairy Commissioner; BILL WINFREY, manager of the Kansas Dairy Association; BOB NORTH, executive secretary of the International Association of Ice Cream Manufacturers; N. E. FABRICIUS, manager of Flambeau Farms Cooperative, Ladysmith, Wis.; BOB GADBERRY, vice president of the Fourth National Bank, Wichita; SAM FINE, chief of the Kansas City District Food and Drug Administration; and ELDON DYE, divisional manager, Fleming and Co., Wichita.

Officers of the merged institutes are G. C. FREEBY, Neosho Valley Cooperative, Erie, president; VINCENT DE COURSEY, De Coursey Creamery, Kansas City, vice president; IVAN VAN NORTWICK, Lawrence, secretary; and HOWARD HURWITZ, Lawrence, treasurer.

D. L. MAC FADDEN, a recent Ph.D. graduate of Kansas State University, has accepted a position with the Staley Milling Co., Kansas City, Mo. His graduate work was in animal nutrition with major work in dairy husbandry.

The Bulk Milk Tank Drivers' School was held the first week in May. The one-day event was sponsored cooperatively by the Kansas State Board of Agriculture, the Kansas State Board of Health, and Kansas State University.

Purpose of the school is to teach rules and regulations regarding operation of bulk milk tank trucks, qualifications and duties of haulers, sanitation principles, sampling methods, and practice in judging milk flavors.

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Beatrice Promotes Walker

E. A. WALKER has been promoted to assistant district manager of the Utah-Nevada District of Beatrice Foods Co.

E. A. Walker

A veteran of 23 years in the dairy business in Northern Utah, Walker joined the Salt Lake City plant of Creameries of America as an accountant following his graduation from the University of Utah with a degree in accounting and marketing in 1936. He advanced to chief accountant and office manager, and, in 1947, was appointed assistant to the division manager.

Creameries of America merged with Beatrice Foods Co. in 1953 and Walker was named manager of the Salt Lake City-Orem operations in 1954.

Walker, who maintains headquarters in Salt Lake City, is a director of the Utah Dairy Council and a member of the Salt Lake City Chamber of Commerce, the Salt Lake Exchange Club, and the Fort Douglas Club.

L. C. SWEARINGEN, veteran of 30 years of experience in food distribution and merchandising, has been promoted to manager of the Beatrice Foods Co. plant in Pueblo, Colo.

Acting manager for the last six months, Swearingen, a native of West Virginia, joined Beatrice Foods in February, 1945, as an advance salesman for the Pueblo plant. He transferred to retail sales and credit supervision when the company entered the fluid milk business in Pueblo. Subsequently, he was promoted to assistant manager, a post he held for several years until he was named acting manager.

Bohstedt Retires from So. Ill. U.

GUSTAV BOHSTEDT, visiting professor of animal industries at Southern Illinois University since September, 1957, has retired for the second time. Bohstedt has left SIU and returned to his home in Madison, Wis. He first retired in 1957 as head of the Animal Husbandry Department at the University of Wisconsin where he had been on the faculty for nearly 35 years.

A native of Guissau, Germany, Bohstedt had a distinguished career as a teacher, researcher, and writer in the field of animal husbandry and is internationally known for his work in animal nutrition. At SIU he taught animal

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husbandry courses and advised the School of Agriculture on developing graduate work in its department of animal industries.

He received his bachelor's, master's, and doctor's degrees at the University of Wisconsin. He also has taught at Iowa State College and was chief of the animal industry department at the Ohio State Experiment Station for seven years in the early 1920s.

Bohstedt is a member of the American Society of Animal Production, the A.D.S.A. and the American Association for the Advancement of Science. He has been honored by a number of professional and semi-professional organizations in the field of feeding and animal nutrition, and has written for a number of farm journals and experiment station research bulletins and circulars.

Borden Opens New Florida Plant

The Borden Company will enter the fluid milk business in Jacksonville, Fla., through the acquisition of the processing and distributing facilities of the Dinsmore Dairy Company, Inc., of that city.

Started as a dairy farm in 1910, Dinsmore Dairy in 1933 established a processing and distributing plant at 660 King Street, Jacksonville, which presently operates about 25 milk routes and a small ice cream business. V. C. JOHNSON, founder-president of the dairy, and his sons, EARL A. and CHARLES, will continue to own and operate the family farm, which is one of the seven large farms supplying the dairy plant.

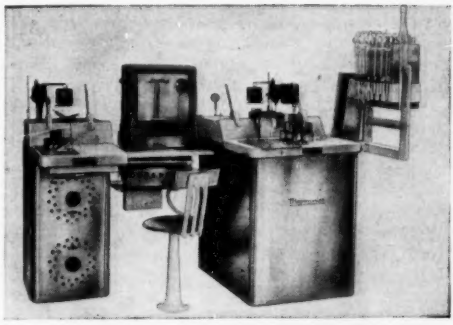
W. J. BARRITT, JR., Tampa, Chairman of Borden's Florida District, said that a modernization program would be started shortly. Through this program, the plant capacity will be increased 25 per cent and improvements made to permit the introduction of Gail Borden Signature Quality milk at a later date.

Through this acquisition, Borden milk will be available in all of the principal cities of Florida. Up to the present, Borden's has operated only an ice cream distributing station in Jacksonville.

DISA Honors Lucas

L. N. LUCAS, vice president of The Bastian Blessing Company, Chicago, became the eleventh person to receive the Honor Plaque awarded for outstanding service to Dairy Industries Supply Association, March 19.

Mr. Lucas first became active in DISA affairs in 1946 as a member of the Exposition Credentials Committee, of which he subsequently served as vice chairman or chairman for many years. Elected to the DISA Board in 1949, he has served as chairman of the Exposition Committee which staged the 1952 Dairy Industries Exposition in Chicago. Other committees in DISA on which he has actively served include the Membership Committee, the Executive Com-



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DISA's Honor Plaque is presented only on occasions when it is deemed highly merited by DISA's Board. In DISA's 40 years, only 11 plaques have been struck.

Eight recipients of the plaque are living. Besides Mr. Lucas they are: R. D. BRITTON, Wisner Manufacturing Corporation; GORDON LAMONT, The Nestle Company, Inc.; H. L. MILLER, Chester-Jensen Company; R. E. OLSON, Taylor Instrument Companies; ROBERT ROSENBAUM, David Michael & Co., Inc.; G. E. WALLIS, The Creamery Package Mfg. Company; and R. L. YOUNG, former DISA Treasurer, now an industry consultant.

Plaque recipients now deceased are: J. H. MULHOLLAND, who was DISA's Honorary President for Life; K. L. WALLACE, A.P.V. (Canada) Ltd.; and EARL WEED, Foote & Jenks, Inc.

New York Approves Revision of Milk Sections of Sanitary Code

The New York Board of Health has approved "in principle and for the time being" revision of the milk sections of the Sanitary Code which do not substantially change existing regulations.

In a statement explaining Board action on the milk sections of the Sanitary Code, it declared:

"The Board has agreed that it will give further study to a number of Code requirements, which in the light of current knowledge and the best modern public health practices, may no longer be necessary.

"Elimination of such provisions now, the Board agreed, may substantially affect economic trade and labor practices in the milk industry. For that reason, the Board was not willing to eliminate them without further study and consideration."

The Board has also approved "in principle and for the time being" the existing regulations of the ice cream and frozen desserts sections of the Code.

The milk sections of the Code which the Board has approved, while not substantially changing existing regulations, contain several new provisions from the protection of the public health. Those additional sections are listed in the Board's statement.

The first of these is a requirement that the pasteurization temperature be raised in order to assure that the Rickettsia of Q fever, that is the microorganism which causes Q fever, will be destroyed. Q fever can be transmitted to man from animals. None has ever been found in either man or animals in New York and thus this preventive measure is wholly precautionary. Q fever is a mild disease, characterized chiefly by headache.

Another provision specifically bans "as unfit for human consumption" all milk which contains penicillin or other antibiotics. This is in line with recent recommendations of the United States Public Health Service. Antibiotics get into milk when cows are treated with them. If milk which contains penicillin is consumed by persons allergic to penicillin, it can cause harmful reactions, ranging from mild to severe.

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Dairy Technology Societies

Central Illinois—W. K. MOSELEY, W. K. Moseley Laboratory, Indianapolis, Ind., spoke at the April meeting on the topic "Problems of Concern to the Control Laboratory."

Kansas City Area—CARROLL MORRIS, owner of Fashion Farms, Meservey, Ia., spoke at the March meeting. Mr. Morris owns a farm and operates a large "milk factory" where neighboring farmers send their cows to be milked. He does not own most of the cows kept and milked on his farm.

Atlanta—P. H. TRACY, University of Illinois, Urbana, spoke at the April meeting and reported the first results of his recent study of automation in the dairy industry.

Kansas—NEIL ANGEVINE, Meyer-Blanke Co., St. Louis, Mo., was the featured speaker at the March meeting. His topic was "Timely Tips on Uniform Cottage Cheese and Other Cultured Products." Ladies night will be featured at the May meeting.

Ohio—D. A. SEIBERLING, consultant, Klenzade Corporation, Beloit, Wis., was the main speaker at the April meetings of the four Ohio Dairy Technology Societies. The Societies are Maumee Valley, Northeastern Ohio, Central Ohio, and Cincinnati. Mr. Seiberling's talk was on "CIP Cleaning in the Dairy Plant."

Detroit—"Newer Methods of Milk Procurement as They Affect the Bacteriological Aspects of Milk," was the topic of P. R. ELLIKER, head of the Dept. of Bacteriology at Oregon State College. He was speaking at the April meeting of the Society.

Michiana—O. A. SWANK, executive secretary of the American Dairy Association, and AL ERICKSON, head of the ADA merchandising department, were main speakers at the April meeting. Their topic was "Dairy Advertising."

Central Michigan—W. K. MOSELEY, Moseley Laboratory, Indianapolis, Ind., spoke at the April meeting of the Society. His topic was "Laboratory Guides to Dairy Operating Procedures."

Minnesota—C. F. WEINREICH, research manager of the Cherry-Burrell Co., Cedar Rapids, Ia., was the speaker at the April meeting. His topic was "Future Planning, New Equipment, New Products."

Philadelphia—"The Future Utilization of Dairy Products," was the topic of D. V. JOSEPHSON, Pennsylvania State University, at the April meeting. Dr. Josephson is head of the Dept. of Dairy Science at Penn State University.

Metropolitan—E. O. HERREID, editor of the Journal of Dairy Science and Professor of Dairy Technology at the University of Illinois, spoke at the April meeting. His topic was "Ultra High Pasteurization of Dairy Products."

North Carolina—P. H. TRACY, University of Illinois, spoke on the status of automation in the dairy industry. His talk featured the April meeting.

Tri-Cities—I. A. GOULD, Dairy Department, Ohio State University, spoke at the April meeting. His topic was "Efficiency Is Our Goal."

Tri-State—A tour of the Kieckhefer Paper Plant, Harmony, Pa., was the featured event at the April meeting of the Society. The tour offered members a look at the complete formation of a paper bottle.

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SIZE MEMORY OF CASEIN COLLOID PARTICLES

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SUMMARY

Fractions of the casein colloid of fresh milk prepared by differential centrifugation, followed by dialysis of each fraction against sodium barbiturate buffer, have been shown by electrophoretic analysis to have the same ratio of α- to β-casein and, by ultracentrifugal analysis, one major component with a sedimentation (S) value of 5.5×10^{-13}. Other components, present in lower concentrations, appear both in the electrophoretic and in the ultracentrifugal patterns. These sodium caseinate preparations, when redialyzed against skimmilk, became opaque and resembled milk. Ultracentrifugal analysis gives S values in the same range as in the original milk. Moreover, the fractions with the larger S values in milk have the larger S values in the reaggregated colloids, ranging from 100 to about 2,000 in the original milk and from 100 to about 400 in the reaggregated colloids.

The state of aggregation of the casein in milk is of interest because of its bearing on milk and milk product stability problems. In its natural state, milk contains casein colloid particles in a range of sizes (1). It is known that substitution of sodium for calcium ion in the casein complex of milk results in reduction of these sizes and the production of a translucent solution of sodium caseinates (8).

The average particle size of the sodium caseinates, as determined by ultracentrifugation, is many times smaller than the average particle size of the calcium caseinate complex existing in the original milk (4).

It is also known (5) that these relatively low molecular weight sodium caseinates aggregate by the readdition of calcium ion to the system. It was felt that the ideal system in which to study this reaggregation would be one containing all of the components of skimmilk except calcium caseinate. Such a system was here nearly obtained by dialysis of sodium caseinates against relatively large volumes of fresh skimmilk.

The purpose of this study was to determine whether the average particle size of the original colloid fraction affected the sizes of aggregates produced by the final redialysis against skimmilk.

METHOD AND MATERIALS

The general procedure used was as follows: A single skimmilk sample was fractionated, in a preparative centrifuge, into a series of casein colloid fractions of various sizes, represented by average S values (where $S = s \times 10^{-13}$ cm/sec per unit centrifugal field), ranging from 180 to 1,850.[3] Each fraction was then

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[3] S values referred to milk, i.e., corrected to the viscosity of skimmilk at the concentration used. The average viscosity of the starting skimmilk was 1.70, relative to water at 20° C.

dialyzed against sodium barbiturate buffer, to replace the bound calcium with sodium. This sodium caseinate preparation was then dialyzed against fresh skimmilk to bring about reaggregation. Thus, there were three states of aggregation possible for each fraction: *(1)* the initial state as present in raw milk; *(2)* the state after dissociation by sodium barbiturate dialysis, and *(3)* the state after reaggregation by dialysis against skimmilk. The effect of each treatment was studied by ultracentrifugal analysis.

Bowl rotor fractionation of the casein colloid. The casein colloid of milk was fractionated by differential centrifugation as follows: A milk sample, collected from a single animal, was centrifuged at a known centrifugal force for 1 min. A small portion of the colloid was thus deposited on a celluloid liner placed in the annulus of the rotor. The rotor was stopped and the deposit removed. The supernatant, now containing most of the particles smaller than those just deposited, was returned to the rotor and the sample centrifuged again, under the same centrifugal force, but for a longer time. In this fashion, a series of deposited fractions containing progressively smaller particles was obtained.

The centrifuge used was an air-driven, air-floated bowl rotor of 70-ml. capacity and 5.5-cm. average radius (2, 3). It was operated at 29,700 r.p.m. for all experimental fractionations.

Data obtained from the fractionation of a typical casein complex from skimmilk with this technique, and the ultracentrifugal analysis of each fraction, are shown in Table 1. Sedimentation constants were determined on 2% solutions of the calcium caseinate colloid. These constants were unchanged after the solutions had been held for seven days at 4° C.

TABLE 1

Bowl rotor fractionation of casein complex from skimmilk at 29,700 r.p.m.

Fraction	Cent. time	Sedimentation constant
	(min.)	*($s_{20,w}$)*
1	1.0	980
2	3.8	870
3	10.8	640
4	60.0	230

Washing of casein colloid fractions. In several experiments, the deposits of casein colloid were washed before dialysis against buffer. In preliminary experiments it was found that washing of the deposit with water produced a more homogeneous fraction, as seen in the ultracentrifuge patterns of the resuspended samples after each washing. Each washing produced a more nearly uniform particle size; however, no further sharpening of the ultracentrifuge peaks was observed after the third washing.

The washing procedure was carried out as follows: Each particular deposit was resuspended in a volume of liquid equal to the volume of skimmilk originally centrifuged. This suspension was then centrifuged for the same time and at the same speed as the skimmilk from which the deposit was obtained. The washings were discarded. Final dissolution of the deposit was accomplished by gently

rotating an Erlenmeyer flask containing the protein, the solvent, and small glass beads for 30 min. at 4° C. A uniform suspension was obtained with no visible precipitate. All centrifugations and washings were done at 20° C.

Washing was done either with distilled water or with milk serum. All of the fractions from one series, however, were given identical treatment; that is, fractions containing particles of different sizes from one series of centrifugations were all washed with water, or were all washed with serum. Milk serum was prepared by centrifugation of a portion of the skimmilk used for micelle fractionation. The centrifuging time for serum preparation was 90 min. The procedure produced a clear, straw-colored solution containing less than 5% of the original casein.

Sodium barbituate dialyses of casein micelles. The casein colloid fractions were dialyzed against sodium barbiturate buffer (pH 8.4, ionic strength 0.10). A sample volume of 20 ml. was dialyzed for 48 hr. against four changes of 1 liter each of buffer at 4° C. in a rotating dialyzer. An aliquot from the dialysis bag of each sample was then analyzed at 59,780 r.p.m. in the ultracentrifuge.

Skimmilk dialysis of sodium caseinate. At the start of ultracentrifugal analysis of each dialyzed sample, an aliquot of the sample was placed in a dialysis bag and dialysis started against fresh skimmilk. Samples were dialyzed against six to ten changes of 2 liters each of skimmilk during six to 16 days. One drop of toluene was placed inside the dialysis bag and one drop in each 2-liter volume of dialysate. Dialysis was at 4° C. At the end of the dialysis, an aliquot of the sample was removed from each dialysis bag and analyzed in the ultracentrifuge (average rotor) at 10,510 r.p.m. To establish the complete conversion of the sodium caseinates to an aggregated form, an aliquot from each sample was also centrifuged at 59,780 r.p.m. and examined for low-molecular weight components. No components in the size range of the unaggregated sodium caseinates were found. To determine whether equilibrium aggregation had been reached, a second aliquot of the aggregated sample was allowed to dialyze against skimmilk for 3 to 7 additional days and again ultracentrifuged. No change was detected.

Ultracentrifugation. The Spinco Model E Ultracentrifuge was used for all sedimentation velocity experiments. Sample temperatures for ultracentrifugation varied between 20 and 25° C. The size range of particles obtained from preparative centrifugation was measured on 2% protein solutions in the solvent used for the washings. A speed of 10,510 r.p.m. was used for these analyses.

The relatively low-molecular-weight sodium caseinates were centrifuged at 59,780 r.p.m. The reaggregated, micelle-like particles resulting from skimmilk dialysis of sodium caseinate were centrifuged at 10,510 r.p.m.

Sedimentation constants were calculated using the increment method (6) and reduced to the viscosity of water or of milk at 20° C.; they were also corrected for adiabatic effects for those experiments carried out at 59,730 r.p.m. (7).

RESULTS AND DISCUSSION

It was found that washed calcium caseinate complex fractions of varying size, prepared by differential centrifugation, were converted by dialysis against

sodium barbiturate buffer to identical solutions of sodium caseinate (as seen in ultracentrifuge patterns), regardless of the size range of the original casein complex micelles (Figure 1). However, when these fractions were reaggregated by dialysis against skimmilk, it was found that the particle sizes of the resulting aggregates were dependent upon the average particle size of the original casein colloid fractions; that is, sodium caseinate made from large casein complex particles aggregated to form large particles, whereas sodium caseinates made from small casein colloid particles aggregated to form small particles when dialyzed against skimmilk. The casein in these colloid fractions retained some sort of "memory" factor or factors causing it to reaggregate to a a predetermined extent in the presence of the dialyzable components of milk.

It was also found that the different casein complex fractions which were not washed, or were washed with milk serum, showed quite different ultracentrifuge patterns after dialysis against sodium barbiturate than those of colloids which were washed with distilled water. Since the washings were not analyzed ultra-

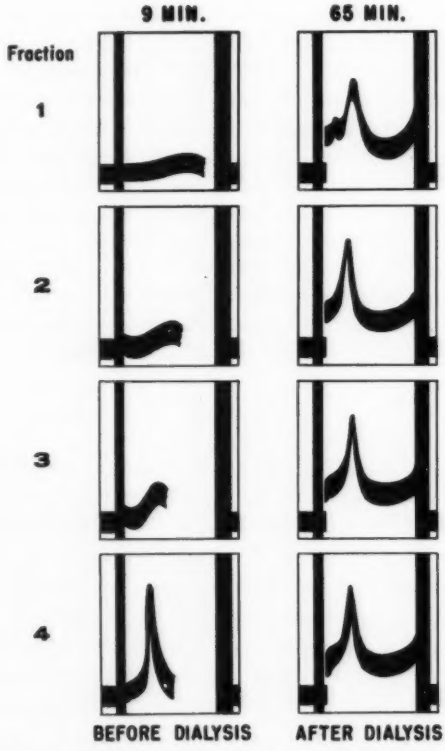

FIG. 1. Ultracentrifuge patterns of centrifugally fractionated cuts of casein colloid from skimmilk; before and after dialysis against sodium barbiturate buffer; sedimentation is from left to right.

centrifugally, it can not be stated whether one component is being converted to another in the sodium caseinate system or whether a micelle protein is being washed out preferentially.

The average sedimentation constants obtained for sodium caseinates were found to be reasonably reproducible, whether the casein complex used as starting material was water-washed, serum-washed, or not washed at all (Table 2).

Also shown in Table 2 are the average sedimentation constants of the major components of casein solutions, made by dialyzing sodium caseinate preparations against skimmilk. Blanks in the data for sodium caseinates indicate the absence of the particular components. Two samples of reaggregated colloid were lost because of bacterial contamination. It appears that reaggregation of sodium caseinates by skimmilk dialysis occurs to an extent dependent upon the original particle size of the casein complex fraction used as starting material. After

TABLE 2

Sedimentation constants of sodium caseinates in sodium barbiturate buffer prepared from various calcium caseinate fractions [a]

Casein complex $s_{20,w}$	Sodium caseinates $s_{20,w}$ / Relative concentration [b]				Dialysis aggregate $s_{20,w}$
"A" 980	1.3/39	4.4/25	6.0/25	9.3/11	400
870	1.1/14	4.3/35	5.3/49	8.0/2	280
640	1.1/16	4.2/28	5.6/34	8.4/22	130
230	1.1/8	4.0/33	5.5/49	8.0/70	116
"B" 910	2.1/28	4.7/48	—	10.0/24	—
390	2.4/34	4.4/30	—	9.2/36	320
120	1.3/45	3.1/37	—	8.9/18	120
"C" 2,430	1.0/21	3.6/76	6.6/3	—	350
2,240	1.1/12	3.3/35	4.0/3	—	—
1,530	1.2/14	3.4/86	—	—	230
1,010	1.1/14	3.5/86	—	—	170
300	1.3/14	3.4/86	—	—	140

[a] "A" samples were not washed and were dissolved in water. "B" samples were washed with milk serum and dissolved in serum. "C" samples were washed thoroughly with water and dissolved in water.

[b] Relative per cent concentrations corrected for radial dilution.

seven days, the rate of reaggregation was essentially zero. Further dialysis did not measurably change the sedimentation constant of the main component of the aggregate.

It is also seen (in Table 2) that washing of the casein complex with water removed from the system some factor or protein which later appears as the S = 9 to 10 component of sodium caseinate in ultracentrifuge patterns made from unwashed calcium caseinate. If the casein colloid preparation is smaller than S = 2,000, washing with water also removes from the system some factor or protein which appears later as the S = 4 to 6 component in ultracentrifugal patterns of sodium caseinates made similarly.

The return of sodium caseinate particle size to the particle size of the original micelle by reaggregation is most nearly complete in serum-wash experiments, less so in the experiments involving no washing, and least evident in water-wash experiments.

CONCLUSIONS

The distribution of sodium caseinates found in preparations of casein made by replacing calcium with sodium in various size fractions of casein micelles is dependent upon the method of preparation.

Washing casein colloid preparations with water removes some factor or protein causing the loss of sodium caseinate with sedimentation constants of S = 6 and S = 9. Washing casein colloids with milk serum causes the disappearance of a S = 6 component from the resulting sodium caseinate system. It can not be determined from the present data whether missing sodium caseinate components are converted to other components or are preferentially removed from the micelle system during the washing of the deposit.

Sodium caseinates aggregate when dialyzed against skimmilk to a size range dependent upon the size range of the original micelles from which the sodium caseinates were made. Reaggregation of casein is dependent upon factors other than calcium ion concentration alone. That is, the protein itself, once synthesized, or once complexed, retains properties affecting its future aggregation and dissociation.

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ISOLATION OF MILK PHOSPHOLIPIDS AND DETERMINATION OF THEIR POLYUNSATURATED FATTY ACIDS[1]

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SUMMARY

Several methods were compared for recovery and purification of mixtures of phospholipids from dried buttermilk. Samples were analyzed for phosphorus, nitrogen, and unsaturated fatty acids. Evidence was obtained that phospholipids purified by repeated acetone precipitation are not representative of the original milk phospholipids. An improved procedure was developed to extract the lipids from buttermilk with ethanol, ether, and chloroform. Most of the phospholipids were separated from the other lipids by precipitation with acetone. Acetone-soluble phospholipids were recovered by chromatography with silicic acid columns. The two fractions were combined and nonlipid contaminants were removed by washing. In an alternative procedure, the lipids were extracted from buttermilk with methanol and chloroform before the foregoing method was used to isolate the phospholipids.

The amounts of unsaturated fatty acids were estimated from spectrophotometric data and iodine values. A representative sample of milk phospholipids (3.72% phosphorus, 2.23% nitrogen, iodine value 53.8) contained 26.3% monoene, 1.5% conjugated diene, and 5.8, 2.4, 1.3, and 1.5% nonconjugated diene, triene, tetraene, and pentaene acids, respectively. Percentages of each of these acids in the total fatty acids (with corresponding percentages for milk fat from the same source given in parentheses) were: 39.8 (32.1), 2.3 (1.4), 8.8 (1.6), 3.6 (0.9), 2.0 (0.3), and 2.2 (0.2), respectively. The phospholipids contained 41.3% saturated fatty acids and the milk fat 63.5%. These data show that there was a selective concentration of nonconjugated polyunsaturated acids in milk phospholipids.

The lipids of milk include about 1% phospholipids, most of which are associated with milk proteins and partly concentrated at the surface of the fat globules. Many investigators (1, 15, 16, 33) have estimated the amounts of phospholipids in milk and milk products by determining the phosphorus content of extracts obtained by various methods, including the Mojonnier modification of the Röse-Gottlieb procedure. In several studies (12, 17, 18, 19, 25, 26, 31, 32) phospholipids were actually separated from the other milk lipids and analyzed. However, these samples were not completely representative of the original phospholipids, except in the recent study by Rhodes and Lea (25). The reason is the difficulties involved in the extraction and purification of phospholipids (23). For example, purification procedures that depend on repeated precipitation of phospholipids with acetone, without recovery of those phospholipids soluble in acetone, result in selective losses.

The Mojonier procedure has often been used for extracting lipids. Although this method does not extract significant amounts of inorganic phosphorus, it may not extract all the individual phospholipids, including lecithins, cephalins,

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[1] A preliminary report on this research was presented at the 53rd Annual Meeting of the American Dairy Science Association, Raleigh, N. C., June, 1958. Some of the data in the published abstract are incorrect. The correct data are in this paper.

and sphingolipids (13, 24). Furthermore, large volumes of solvents are required for isolating even a small sample of phospholipids. For these reasons, other methods of extraction have been employed to recover phospholipids from buttermilk (25, 26, 28, 31) or from the fat-free serum of butter (12, 17, 24), each of which contains higher proportions of these constituents than milk does. Since buttermilk includes the smaller fat globules that may escape incorporation into the butter granules, as well as membrane constituents from the larger fat globules, the phospholipids it contains would not be expected to differ from those of cream. Rhodes and Lea found that the phospholipids of buttermilk and skimmilk are similar in composition, and concluded that the phospholipids of buttermilk can be considered representative of those of whole milk.

The unsaturated fatty acids of milk phospholipids are of special interest because they are generally believed to be involved in the early stages of oxidative deterioration (20). Published values for these acids, obtained by distillation of their esters, are in conflict. Kurtz *et al.* (19) reported that the unsaturated fatty acids of milk lecithin-cephalin were 70.6% oleic acid and 6.3% C_{22} acid with four double bonds. In contrast, Hilditch and Maddison (12) found that the component acids of two milk phospholipid samples included, respectively, 32.5 and 23.5% oleic acid, 6.4 and 0% octadecadienoic, and 7.8 and 12.8% $C_{20\text{-}22}$ unsaturated acids.

Improved methods recently have become available for isolating, purifying, and analyzing different lipids. In 1952, Borgström (2) reported that the phospholipids of rat serum could be separated from the neutral fat by chromatography with silicic acid columns. Since then, his technique has been successfully applied in separating phospholipids from other sources (22). Folch *et al.* (4) developed a purification procedure in which an extract of crude lipids from animal tissues in a 2:1 mixture of chloroform and methanol is mixed with one-fifth volume of water. On standing, two phases are obtained, with the nonlipids going to the upper phase. Lea and Rhodes (21) eluted egg-yolk phospholipids from a cellulose column with chloroform equilibrated with ethanol and water to remove amino acids. Hawke (9) also used cellulose columns to purify muscle and liver phospholipids, but replaced the chloroform-ethanol-water eluant by chloroform equilibrated with water. Improved spectrophotometric methods (14) have been developed to determine polyunsaturated fatty acids in lipids extracted from fluids and tissues of animals and plants. The present investigation compares the compositions of milk fat and milk phospholipids prepared by different methods, with particular reference to their component polyunsaturated fatty acids.

EXPERIMENTAL PROCEDURE

Materials. Three lots of spray-dried buttermilk were used as source of phospholipids. Lot A was a typical commercial product, whereas Lots B and C were specially prepared with low heat treatments. Buttermilk for Lot A was preheated to 185° F. for 30 min. before it was condensed in a double-effect (160 and 120° F.) evaporator and dried. Buttermilk for Lot B was

preheated to 143° F. and condensed at 125° F. in vacuum prior to drying in the departmental pilot spray drier. The powder was collected in small lots and cooled as soon as possible. Buttermilk for Lot C was heated to 161° F. in a H.T.S.T. pasteurizer, condensed in a double-effect (155 and 102° F.) evaporator, dried, and immediately sacked. Respective total lipid contents (Mojonnier method) of Lots A, B, and C were 4.77, 11.30, and 4.52%, and moisture contents (toluol distillation method) were 5.80, 2.82, and 3.40%.

Milk fat MF1 was prepared by churning pasteurized cream, melting the butter granules, and filtering the clear fat at 50° C. Milk fat MF2 was obtained by chromatography during the preparation of phospholipid PL7 from Lot C buttermilk.

Pure synthetic L-α-(dimyristoyl)-lecithin [2] was used as a control in determinations of phosphorus, nitrogen, and polyunsaturated fatty acids.

Analytical methods. Pentane and iso-octane were purified by percolation through silica gel (5). Acetone, *n*-butanol, chloroform, ethyl ether (peroxide-free), and methanol were reagent grade.

The milk phospholipids were protected against oxidation during isolation by being kept in solution and by avoiding exposure to light and oxygen as far as possible. All samples were stored in chloroform under nitrogen at 0–5°C., and analyzed within a few days of preparation. When freed of solvent, acetone-insoluble phospholipids were white and acetone-soluble light amber.

Weights of lipid fractions were calculated from weights of residues obtained from aliquots of the lipid solutions. All analytical data reported are averages of at least two determinations. Lipid phosphorus was determined by a modification (30) of the method of Harris and Popat (8), and total nitrogen by a semimicro-Kjeldahl method. Iodine values were obtained by the Hanus procedure, using 100-mg. samples and 15.0 ml. of reagent.

Conjugated fatty acids were determined by the method of Brice *et al.* (3) with data corrected for absorption by extraneous compounds. Methanol containing 0.8 or 8.0% chloroform, depending on dilution, was used as solvent for the phospholipids and iso-octane for the fats.

Nonconjugated fatty acids were usually determined on 70-80 mg. of sample isomerized at 180° C. for 15 min. in 11.0 g. of 21% potassium hydroxide-glycol solution, according to the conditions of Herb and Riemenschneider (10). Their micro method (11) was employed for the dimyristoyl lecithin. With both procedures, the required amount of lipid in 5 ml. or less of solvent was transferred with a pipette to an isomerization tube, which was placed in a water bath at 60° C., and the solvent was removed with a stream of nitrogen. One milliliter of methanol was added and then evaporated in the same way to ensure the removal of any traces of solvent that might give rise to extraneous absorption. The required amount of 21% KOH-glycol (11.0 g. for macro and 5.0 g. for micro method) at 180° C. was added volumetrically to each isomerization tube with a tuberculin syringe fitted with a 4-in. No. 18 S.S. needle. Then the nitrogen-distributing head was replaced, the tube shaken 5 sec., and placed in

[2] LaMotte Chemical Products Co., Chestertown, Md.

the isomerization bath at 180° C. Shaking was repeated twice more, at 1-min. intervals, and total heating time was 15 min. Agreement in results was satisfactory when the macro and micro methods were compared using the same samples of milk fat and milk phospholipids.

In calculating the amounts of nonconjugated acids, the absorptivity at 233, 268, 315, and 346 mμ that was present before isomerization was subtracted from that found after isomerization. The equations under "*(c)*" of Herb and Riemenschneider's micro method (11) were used. Total monoethenoid acids were calculated as per cent of oleic acid from the difference between the iodine value and unsaturation accounted for by the polyunsaturated acids.

Chromatography. A 2:1 mixture of silicic acid (Mallinckrodt) and celite (100-200 mesh, acid-washed) was employed in the experiments where phospholipids were separated from other milk lipids by chromatography. The silicic acid was heated overnight at 120° C. before use, and the columns were packed either dry or in a slurry with methanol. Before use, the columns were washed with two column-volumes of each solvent, in reverse order to that used in the run.

RESULTS AND DISCUSSION

Isolation of milk phospholipids. In the present study, several methods of preparing phospholipids from spray-dried buttermilk were investigated, and percentages of phosphorus and nitrogen and iodine values for representative samples are given (Table 1).

TABLE 1

Analyses of milk fat, dimyristoyl lecithin, and milk phospholipids

Sample[a]	P	N	Iodine value (Hanus)	Unsaturated fatty acids					
				Conjugated	Nonconjugated				
				Diene	Monoene[b]	Diene	Triene	Tetraene	Pentaene
	(%)			(%)					
MF1	.....	.....	30.60	0.71	24.98	1.33	0.91	0.25	0.26
MF2	.....	.....	35.21	1.24	29.21	1.49	0.84	0.25	0.21
Dimyristoyl lecithin	4.46	2.10	0.00	0.00	0.00	0.03	0.00	0.01	0.01
PL1	3.57	1.64	49.12	1.02	9.81	6.27	3.10	2.14	2.84
PL2	3.34	1.84	41.11	1.22	17.78	4.69	1.85	1.15	1.37
PL3-EI	3.10	2.58	28.34	0.44	17.17	1.94	1.24	0.59	0.80
PL3-ES	3.56	2.01	56.45	1.76	27.68	5.16	2.51	1.50	1.78
PL4	3.03	2.18	45.80	1.38	21.92	4.03	2.04	1.22	1.65
PL5	3.41	3.85	50.46	1.62	25.23	4.58	2.01	1.24	1.72
PL6-AI	3.41	2.20	49.10	1.15	24.29	5.08	2.15	1.28	1.45
PL6-AS	3.36	2.02	58.80	2.39	24.41	6.30	2.39	2.02	1.95
PL7	3.72	2.23	53.75	1.53	26.27	5.80	2.40	1.32	1.45
PL8	3.44	2.10	52.22	1.50	24.53	5.30	2.09	1.47	1.80

[a] See text for methods used to prepare individual samples.

[b] Calculated as oleic acid from difference between iodine value and unsaturation accounted for by polyunsaturated fatty acids.

Phosphorus and nitrogen percentages can not adequately indicate the composition or purity of samples containing mixtures of cephalins, lecithins, sphingolipids, and possibly other associated lipids, because the different classes of phospholipids have different N:P ratios and contain fatty acids of unknown

molecular weights. However, despite these limitations, such data and iodine values are useful in comparing phospholipids prepared by different methods.

Phospholipid sample PL1, obtained from Lot A buttermilk by *n*-butanol extraction and acetone precipitation, was analyzed as described previously (28). The results showed that *n*-butanol was useful to split phospholipid-protein complexes. Yield and purity of this sample were comparable to those for phospholipids isolated by others from buttermilk (26). Because comparatively high temperatures were required to remove *n*-butanol, with attendant danger of sample deterioration, extraction of phospholipids with other solvents was studied.

Phospholipid PL2, extracted from Lot B buttermilk with a 1:1 ethanol-ether mixture, was purified by precipitation with acetone. Fifty grams of powder was dispersed in 100 ml. of water with a Waring Blendor, and extracted, in turn, with 200-ml. lots of ethanol and ethyl ether. After filtration, the residue was re-extracted with 200 ml. of 1:1 ethanol-ether, and the filtrates were combined. The lipids were removed from the filtrates by extracting, in turn, with 300 ml. of pentane and 100 ml. of 1:1 ether-pentane. After drying the solution with anhydrous sodium sulfate, nearly all the solvent was removed under reduced pressure with a Rinco Rotating Evaporator.[3] The phospholipids were precipitated with 100 ml. of acetone, and further purified by reprecipitating from acetone three times. About 200 mg. of phospholipids were recovered from 50 g. of buttermilk powder. The nitrogen percentage was higher, but the phosphorus content and iodine value were lower for PL2 than for PL1. Sample PL2 represented only about one-third of the phospholipids calculated to be present from the phosphorus content of the lipids extracted by the Mojonnier procedure. In an attempt to improve phospholipid recovery, the use of hot solvents was investigated.

Phospholipids PL3-EI and PL3-ES were, respectively, the ether-insoluble and ether-soluble fractions from a sample extracted from Lot B buttermilk by ethanol, ether, and chloroform. Fifty grams of powder was blended for 2 min. with 200 ml. of 95% ethanol at 80° C. and allowed to stand for 5 min. Then 200 ml. of ether at 35° C. was added and the mixture was extracted again by blending for 2 min. After filtration, the residue was re-extracted for 2 min. with 200 ml. of chloroform at 60° C., and was again filtered. Filtrates from four 50-g. lots of buttermilk were combined, and the solvents evaporated below 60° C. with the Rinco Evaporator. When nearly dry, the lipid residue was extracted with 400 ml. of a 50:50:1 mixture of ether-pentane-ethanol and dried with 50 g. of anhydrous sodium sulfate. After removing the sodium sulfate and nonlipid material by filtration, the solution was evaporated below 50° C. until nearly dry.

The above extracted lipids were dissolved in 40 ml. of ether, and the phospholipids were precipitated by adding 800 ml. of acetone. After 30 min., the acetone-fat layer was removed by filtration and the phospholipids were washed

[3] A. S. Aloe Co., San Francisco, Calif.

with 50 ml. of acetone. The crude preparation was dispersed in 25 ml. of ether, slowly poured into 300 ml. of swirling acetone at 50° C., and allowed to stand overnight at 22° C. The sample was further purified by reprecipitating from acetone four times, and then dissolved in warm ether and held 48 hr. at 5° C. to obtain ether-insoluble fraction PL3-EI and ether-soluble fraction PL3-ES. The iodine values and percentages of phosphorus and nitrogen are consistent with the observations of Kurtz *et al.* (19), who separated milk phospholipids into an ether-soluble lecithin-cephalin fraction and an ether-insoluble sphingomyelin-cerebroside fraction.

The above extraction procedure gave a higher phospholipid recovery than did extractions carried out at room temperature. However, it is known (23) that acetone does not precipitate phospholipids quantitatively, especially in the presence of large amounts of triglycerides. Therefore, experiments were designed to study the recovery of milk phospholipids from lipid extracts by acetone precipitation and column chromatography. Data from a representative experiment are presented (Table 2).

TABLE 2

Recovery of lipids and lipid phosphorus in purification of milk phospholipids by acetone precipitation and column chromatography [a]

Fraction	Lipid weight		Lipid phosphorus	
	(g.)	*(%)*	*(mg.)*	*(% recovered)*
Acetone precipitation				
BM B lipids	10.45	0.524	54.8	100.0
1st acetone-soluble	8.68	0.054	4.7	8.6
2nd acetone-soluble	0.12	1.46	1.7	3.1
3rd acetone-soluble	0.07	2.20	1.6	2.9
Acetone-insoluble (PL4)	1.50	3.03	45.5	83.0
Total recovered	10.37		53.5	
Acetone precipitation and column chromatography				
(1) BM B lipids	10.45	0.524	54.80	100.0
(2) Acetone-insoluble	1.83	2.73	50.00	91.2
(3) Acetone-soluble	8.36	0.058	4.85	8.8
Chromo. separation of (3)				
(3A) Chloroform eluate	8.04	0.008	0.64	
(3B) Acetone eluate	0.07	0.030	0.02	
(3C) Methanol eluate	0.15	2.12	3.18	5.8
Total recovered	8.26		3.84	
Chromo. separation of combined (2) and (3C)	1.98	2.69	53.18	
(4A) Chloroform eluate	0.22	0.025	0.06	
(4B) Acetone eluate	0.19	0.043	0.08	
(4C) Methanol eluate (PL5)	1.55	3.41	52.86	96.5
Total recovered	1.96		53.00	

[a] Data corrected for aliquots used in analyses.

The total lipids from 200 g. of Lot B buttermilk were extracted by the above hot solvent method. Lipid recovery was over 92% of that obtained by the Mojonnier technique. The phospholipids in one-half of the material were precipitated three times with acetone. Table 2 shows that the acetone-insoluble fraction (PL4) contained 83% of the original total phosphorus and that 15% was lost in the acetone-soluble lipids.

Most of the phospholipids in the other half of the extracted material were recovered by acetone precipitation, and the remainder by chromatography before the combined phospholipids were rechromatographed. After the addition of acetone to the extracted lipids, the insoluble and soluble fractions were recovered. The soluble fraction was applied in 150 ml. of chloroform to a silicic acid–celite column (16 × 3.4 cm.), and the column was eluted with 1 liter each of chloroform, acetone, and methanol. The residue from methanol eluate 3C was combined with the acetone-insoluble fraction 2 and was rechromatographed on a fresh column, using 1,200, 1,600, and 1,600 ml. of chloroform, acetone, and methanol, respectively, for elution.

Table 2 shows that most of the lipid phosphorus in the acetone-soluble fraction 3 was recovered by chromatography. The final methanol eluate 4C, from which phospholipid PL5 was recovered, contained over 96% of the original phosphorus extracted. Therefore, phospholipids prepared by the above procedure should be more representative than those prepared by repeated acetone precipitation. However, Table 1 shows that sample PL5 contained not only a higher percentage of phosphorus than did PL4 but also a higher percentage of total nitrogen. These data, and similar results for other lots of phospholipids prepared in the same way, suggested that amino acids and possibly other phospholipid-protein constituents accompanied the phospholipids isolated by chromatography. Therefore, methods for removing nonlipid contaminants were studied.

The three techniques recommended by Folch *et al.*, Lea and Rhodes, and Hawke were used to purify milk phospholipids. Each decreased the total nitrogen percentage significantly, but the Folch *et al.* procedure gave the greatest increase in phosphorus percentage and required smaller volumes of solvents than did the chromatographic techniques. Furthermore, Hanahan *et al.* (7) found that removal of amino acids by cellulose columns resulted in losses of phospholipids. For these reasons, the method of Folch *et al.* was subsequently employed to purify milk phospholipid samples.

Acetone-insoluble and acetone-soluble phospholipids were next prepared so that their composition could be compared. After extracting the lipids from 400 g. of Lot C buttermilk by hot ethanol, ether, and chloroform, 2.7 g. of sample PL6-AI (Table 1) was obtained by precipitating the acetone-insoluble phospholipids twice and then washing them once by the above method. The acetone-soluble phospholipids were recovered by elution with methanol from silicic acid–celite columns and were washed once to obtain 1.3 g. of sample PL6-AS. Comparison of phosphorus and nitrogen percentages shows that contaminants were removed from both samples and suggests that the acetone soluble phospholipids were mostly cephalins and lecithins.

To prepare a representative sample of phospholipids, a similar procedure was used, except that the acetone-insoluble and acetone-soluble fractions were combined before they were washed twice. The flow diagram (Figure 1) shows the preparation of phospholipid PL7 and milk fat MF2 from Lot C buttermilk. The yield was approximately 4 g. of phospholipids from 400 g. of powder.

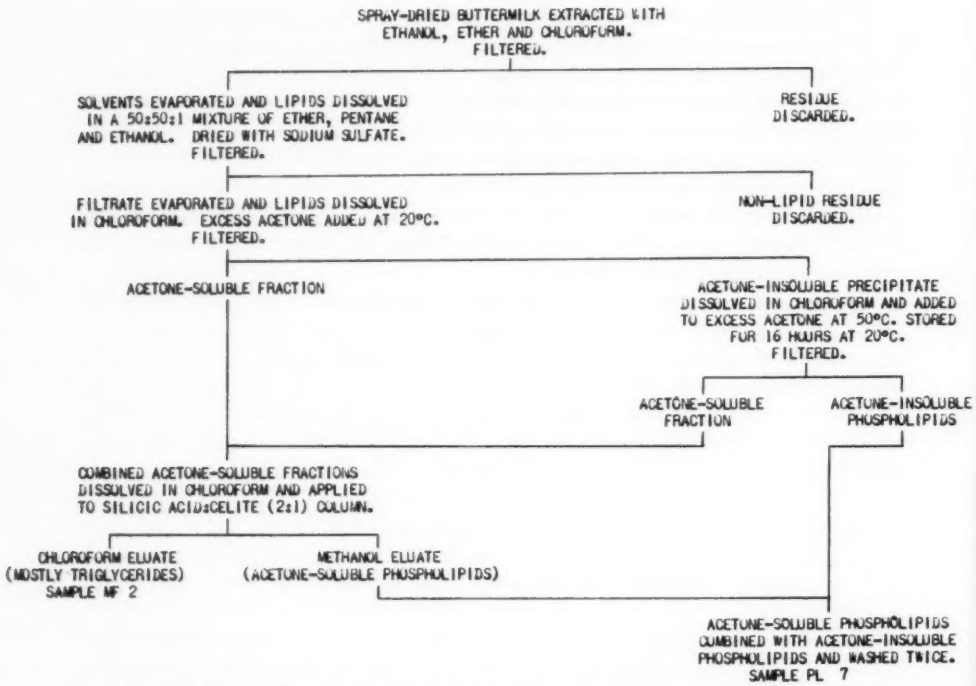
SPRAY-DRIED BUTTERMILK EXTRACTED WITH ETHANOL, ETHER AND CHLOROFORM. FILTERED.
SOLVENTS EVAPORATED AND LIPIDS DISSOLVED IN A 50:50:1 MIXTURE OF ETHER, PENTANE AND ETHANOL. DRIED WITH SODIUM SULFATE. FILTERED.
RESIDUE DISCARDED.
FILTRATE EVAPORATED AND LIPIDS DISSOLVED IN CHLOROFORM. EXCESS ACETONE ADDED AT 20°C. FILTERED.
NON-LIPID RESIDUE DISCARDED.
ACETONE-SOLUBLE FRACTION
ACETONE-INSOLUBLE PRECIPITATE DISSOLVED IN CHLOROFORM AND ADDED TO EXCESS ACETONE AT 50°C. STORED FOR 16 HOURS AT 20°C. FILTERED.
ACETONE-SOLUBLE FRACTION
ACETONE-INSOLUBLE PHOSPHOLIPIDS
COMBINED ACETONE-SOLUBLE FRACTIONS DISSOLVED IN CHLOROFORM AND APPLIED TO SILICIC ACID:CELITE (2:1) COLUMN.
CHLOROFORM ELUATE (MOSTLY TRIGLYCERIDES) SAMPLE MF 2
METHANOL ELUATE (ACETONE-SOLUBLE PHOSPHOLIPIDS)
ACETONE-SOLUBLE PHOSPHOLIPIDS COMBINED WITH ACETONE-INSOLUBLE PHOSPHOLIPIDS AND WASHED TWICE. SAMPLE PL 7

FIG. 1. Flow diagram showing procedure adopted for isolating phospholipid and triglyceride fractions from buttermilk.

Phosphorus and nitrogen percentages are consistent with those corresponding to a mixture of cephalins, lecithins, and sphingolipids.

A few experiments were carried out in which methanol and chloroform were used for the first extraction of the lipids from buttermilk and chloroform alone for re-extraction of the first extract. The remainder of the preparative procedure was similar to that outlined in Figure 1. Analytical data for a representative sample (PL8) are in Table 1. This method gave similar phospholipid yields, but the samples generally contained slightly less phosphorus than those prepared according to the scheme outlined in Figure 1. Although the latter method of preparation is preferred, the methanol-chloroform technique is considered to be a useful alternative technique.

Polyunsaturated fatty acid composition. Table 1 presents the results obtained for all samples, except that any traces found of conjugated trienoic and tetraenoic constituents are omitted. No spectral evidence was obtained to indicate the presence of nonconjugated constituents more unsaturated than pentaenoic. The isomerization conditions (10) employed in this study confirmed the presence of about 0.25% nonconjugated pentaenoic acid in milk fat (29). Percentages of the other nonconjugated and conjugated acids in milk fats MF1 and MF2 are in agreement with values reported in the literature (16).

Figure 2 shows typical ultraviolet absorption spectra of milk phospholipids after alkali isomerization. Spectral positions of the nonconjugated absorption maxima are in agreement with data characteristic of alkali-isomerized methyl esters of linoleic, linolenic, arachidonic, and docosapentaenoic acids (10). However, because animal lipids may contain a complex mixture of polyunsaturated acids, including both positional and geometric isomers (14), the data in Table 1 have been expressed as percentages of diene, triene, tetraene, and pentaene acids present. Less confidence can be placed on the percentages of monoene acid, because they were not determined directly but were calculated. Further-

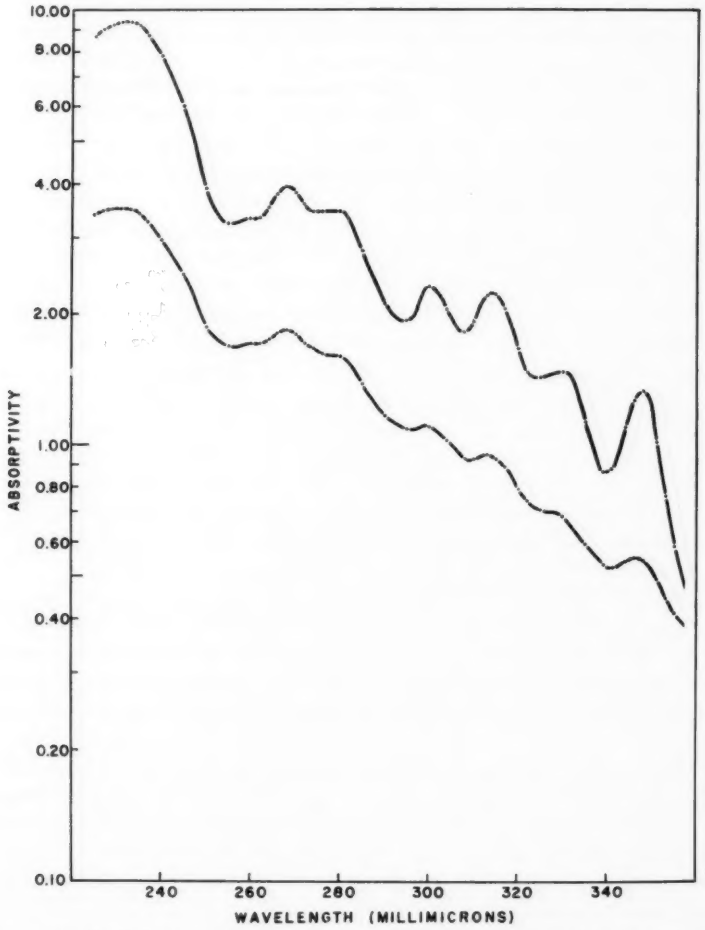

FIG. 2. Ultraviolet absorption spectra of milk phospholipids after alkali isomerization. Upper curve—PL3-ES, lower curve—PL3-EI.

more, if milk phospholipids contain small amounts of C_{12-16} monoethenoid acids, as does milk fat (16), then the monoene values reported may be slightly high. The fatty acid data for dimyristoyl lecithin show that the phosphate-choline part of the molecule did not cause significant absorption in the ultraviolet spectral region, either before or after alkali isomerization. This provides additional evidence that the spectrophotometric method for determining polyunsaturated fatty acids may be applied directly to both glycerophospholipids (cephalins and lecithins) and triglycerides. No evidence was obtained that the method did not measure the small amounts of unsaturated fatty acids that might be present in sphingolipids (sphingomyelins and cerebrosides).

Table 1 shows that mixtures of milk phospholipids extracted from buttermilk are more unsaturated than a typical sample of milk fat or a milk fat prepared from the same source as the phospholipids (MF2). Differences in fatty acid composition among the phospholipid samples can be attributed to differences in source material and/or methods of phospholipid preparation.

The data for fractions PL3-EI and PL3-ES show that the ether-soluble phospholipids contained more of each unsaturated fatty acid than did the ether-insoluble phospholipids (Figure 2). Infrared absorption spectra of films of these two samples had absorption peaks corresponding to mixtures of lecithins, cephalins, and sphingomyelins (6, 27), but the absorption peaks at 6.1 and 6.5 μ, characteristic of amide C=O, were much more pronounced in PL3-EI. These results and the higher N/P ratio of this sample confirm the concentration of sphingomyelins in the ether-insoluble phospholipid fraction. The lower percentages of unsaturated fatty acids in this fraction are consistent with reports (18, 23) that the fatty acids in the sphingomyelins are much more saturated than are those in the glycerophospholipids.

The higher percentage of nitrogen in sample PL5 than in sample PL4 shows that small amounts of extraneous nitrogen-containing compounds (possibly amino acids) were recovered with the acetone-soluble phospholipids. However, the presence of these contaminants would not interfere with determination of the unsaturated fatty acids in this or any of the other samples.

Analyses of fractions PL6-AS and PL6-AI demonstrate that the acetone-soluble phospholipids are more unsaturated than the acetone-insoluble phospholipids. These data also suggest that purification of phospholipid samples by repeated acetone precipitation would result in a selective concentration of sphingomyelins and the more saturated glycerophospholipids in the acetone-insoluble fraction.

Of the results presented in Table 1, the percentage of unsaturated fatty acids in samples PL5, PL7, and PL8 are considered to be most representative of the amounts present in milk phospholipids. Since the individual classes of phospholipids (lecithins, cephalins, and sphingolipids) contain no more than two fatty acids per molecule, compared to three component acids in each triglyceride, the fatty acids of milk phospholipids must include a higher proportion of each unsaturated acid than is shown by percentage of total phospholipid weight. To obtain this information, the amounts of fatty acids in milk fats

MF1 and MF2 were determined. Each fat was refluxed with 10% potassium hydroxide in ethanol and, after extracting the unsaponifiable matter with ether, the hydrolysate was acidified with 20% sulfuric acid, and the fatty acids were recovered by repeated extraction with ether and pentane. The fatty acids were hydrolyzed from phospholipid samples PL7 and PL8 by refluxing before they were recovered, first with 3% ethanolic sulfuric acid and then with 10% ethanolic potassium hydroxide.

Table 3 lists the total amounts of fatty acids recovered from each of the above samples, and the amounts of saturated acids and individual unsaturated fatty acids were calculated as percentages of the total fatty acids. These data show that fatty acids of milk phospholipids included several times the amounts of polyunsaturated components found in the corresponding triglycerides. Furthermore, there was a selective concentration of nonconjugated diene, triene, tetraene, and pentaene constituents in the phospholipids.

TABLE 3
Fatty acid composition of milk fat and phospholipids

Sample[a]	Total fatty acids	Per cent of total fatty acids						
		Conjugated diene	Nonconjugated					Saturated acids[b]
			Monoene	Diene	Triene	Tetraene	Pentaene	
	(%)							
MF1	91	0.78	27.44	1.46	1.00	0.27	0.29	68.76
MF2	91	1.36	32.10	1.64	0.92	0.27	0.23	63.48
PL7	66	2.32	39.81	8.78	3.64	2.00	2.20	41.25
PL8	67	2.24	36.60	7.91	3.12	2.19	2.69	45.25

[a] See text for methods used to prepare individual samples.
[b] By difference.

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DISTRIBUTION OF NATURAL AND ADDED COPPER AND IRON IN MILK [1]

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SUMMARY

A radioactive tracer was used to determine the distribution of copper in milk. For comparison, the distribution of radioactive iron also was determined. The method can readily distinguish the sources (natural or added) of copper or iron.

Marked differences were observed in the distribution of natural and added copper. Naturally occurring copper was concentrated (*ca.* 10–35% of the copper) at the surface of the fat globules; whereas, most of the added copper was uniformly associated with the skimmilk proteins. Only 2–3% of the total added copper was associated with the fat globules. Natural and added copper in association with the fat globules were recovered with the fat globule membrane proteins.

The distribution of natural iron, like natural copper, favored the fat globules, but none of the added iron became associated with the fat globules. Natural iron not associated with the fat globules was more concentrated with the whey proteins than with casein.

Natural copper and iron were not dialyzable. Added copper and iron in the skimmilk were slightly dialyzable, and the amount of unbound metal increased as pH was decreased to 3.0. Added copper associated with fat globules was not dialyzable.

It has long been recognized that copper contamination in milk frequently causes oxidized flavor; more recently, it has been reported (7) that copper occurring naturally in milk is also related to the development of this defect. The nature of copper catalysis of oxidized flavor is not clearly understood. A more complete knowledge of the chemical state and distribution of both natural and added copper in milk would be helpful in elucidating the mechanism for the development of oxidized flavor.

The distribution of added copper in milk has been studied by a number of investigators; however, the data are both incomplete and controversial. Most of the investigators have studied the partition of copper (and in some cases iron) between skimmilk and cream. It has been reported that added copper is preferentially associated with the fat phase of milk and, conversely, that it is primarily associated with the skimmilk.

Rice and Miscall (12) found that added copper was uniformly distributed in milk in accordance with total nitrogen or water content. They concluded that it was ionic. In a similar study, Davies (2) reported that added copper and iron were uniformly distributed in gravity-separated milk, but that the

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copper was concentrated in the cream after the milk had been subjected to centrifugal separation. He concluded that added copper was distributed in proportion to the surface area of the fat globules. On the basis of diffusion experiments, he found that the amount of ionic copper was negligible at the normal pH of milk, but it increased as pH decreased.

Willard and Gilbert (14) reported that copper became concentrated in the cream of milk subjected to contamination from copper-alloy equipment. Miller and Tracy (10) found that, when cream used in a continuous butter-making operation was subjected to heavy copper contamination, most of the copper was recovered in the skimmilk. Hartmann (4) reported that the whey proteins had the greatest affinity for copper.

Van der Waarden (13) studied the distribution of natural and added copper and iron in butter. He found that the triglycerides contained no metals, the proteins contained all of the iron and 50–75% of the copper, the phospholipids contained 25–50% of the copper, and the water was free of metals. He concluded that the natural and added forms of copper and iron were both distributed in a similar manner.

By fractional precipitation with ammonium sulfate, Dills and Nelson (3) isolated from cow's milk a copper-bearing protein with no catalytic activity. The copper was not removable by dialysis at pH 6.6, but was completely removed at pH 3.5.

Allan (1) studied the kinetics of ascorbic acid oxidation in milk and butter serum. He concluded that both added and natural iron and copper were nonionic and were associated with the fat globule membrane. However, added copper was a more effective catalyst than natural copper.

Koppejan and Mulder (8, 11) studied the variation and distribution of natural copper in individual milks. They found that milk was considerably higher in copper content in early lactation than later on, and reported that 4–43% of the total natural copper was associated with the fat globules. Their limited data indicated that added copper and iron do not accumulate at the fat globule surface.

Menger and Mulder (9) reported that added copper became primarily associated with the skimmilk proteins, and that natural copper was primarily associated with the fat globule membrane. They found no correlation of the high natural-copper content of early-lactation milk with the keeping quality of the butter made therefrom, and reported that only added copper catalyzed flavor defects in butter. They also discussed the difficulty in distinguishing the source of copper in milk by present analytical techniques.

In the present investigation a radioactive-tracer technique was employed to facilitate determination of the distribution of copper in milk and to distinguish the source of copper. Some observations are included on the nature of the chemical association of copper and iron in milk. The work was directed primarily toward copper but, for comparison, the distribution of radioactive iron was also determined.

EXPERIMENTAL PROCEDURES

The milks used, primarily from individual cows in the University herd, were selected on the basis of type of feed, stage of lactation, and susceptibility to oxidized flavor (7).

Isotopes. The isotopes used in this study were Cu^{64}, half-life 12.8 hr., and Fe^{59}, half-life 45 days, obtained from the Oak Ridge National Laboratory. Cu^{64} was obtained and used as cupric nitrate, and Fe^{59} as ferric chloride.

To study the distribution of natural copper and iron in milk, the isotope used was diluted with physiological saline solution and administered by slow infusion into the jugular vein over a 2-hr. period. The cow was milked immediately before infusion of the isotope, and the first experimental samples were drawn from the udder 8–12 hr. after the infusion. The milk was fractionated immediately without cooling, and the concentration of the isotope in the various fractions was determined by measurement of radioactivity. The terms natural Cu^{64} and natural Fe^{59} are used to refer to the copper and iron present in milk drawn following infusion of the isotopes.

For experiments on the distribution of added copper and added iron, the isotope was added directly to freshly drawn warm milk at a concentration of 0.2–1.0 p.p.m. (isotope and carrier) and the milk was held at least 2 hr. before fractionation.

Milk fractionation. The scheme used, completely or in part, for fractionation of milk is shown in Figure 1.

Milks were separated at 40° C. (DeLaval Model 518 separator) and the creams were washed four times with 3–4 volumes of distilled water (45° C.).

Casein was removed from the skimmilk both by isoelectric precipitation at pH 4.5 and by supercentrifugation for 2 hr. at 26,000 G with a Servall angle centrifuge (Model SSIA). Casein was redispersed in 0.1 *N* sodium hydroxide in a volume about equal to that of the skimmilk.

Whey proteins were precipitated by saturation with ammonium sulfate and removed by filtration.

The washed creams were cooled for 1–2 hr. in an ice-water bath, and churned in glass bottles by mechanical agitation. The aqueous phase (membrane solution) was removed by decanting. The butter was melted in a hot-water bath and centrifuged for clarification and removal of the aqueous phase (residual membrane solution).

The membrane solution was concentrated either by isoelectric flocculation or by saturation with ammonium sulfate, followed by centrifugation. The concentrated membrane material was fractionated into protein and lipid components by the procedure described by Herald and Brunner (5). The membrane proteins were dispersed in 10% sodium hydroxide and diluted to the original volume of the membrane solution. The membrane lipids were made to the same volume with ether.

Analytical. Radioactivity was determined directly on 2-ml. liquid aliquots with a deep-well scintillation counter (Model S-2B well counter, Model L-2

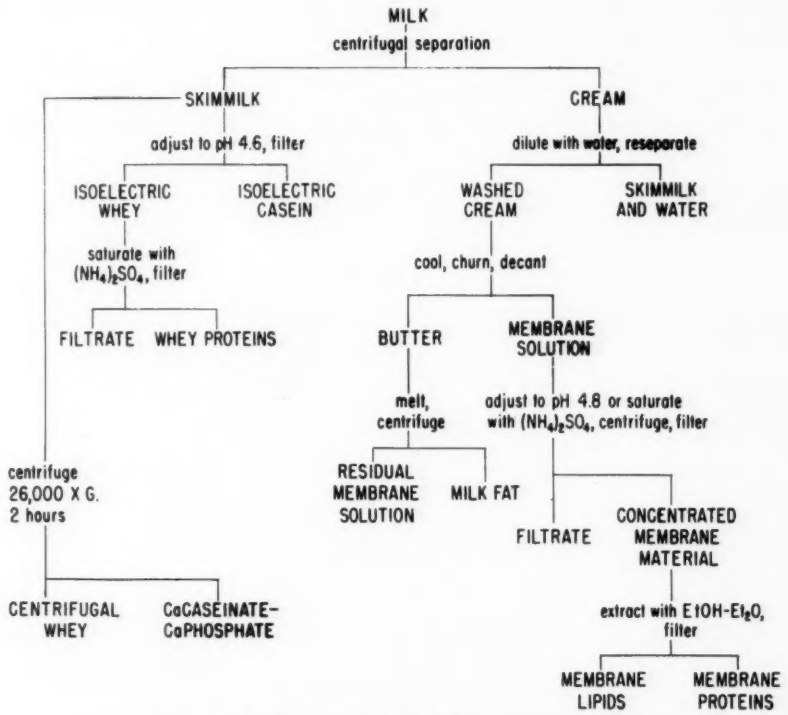

FIG. 1. Flow diagram illustrating fractionation of milk.

counter shield[5]), with a background of 80 c.p.m. (counts per minute). Radioactivity is expressed as c.p.m., corrected for decay and background.

Nitrogen was determined by a semimicro-Kjeldahl procedure, and fat either by the Babcock or by the Mojonnier method.

Dialysis. To determine the dialyzability of Cu^{64} or Fe^{59}, 5-ml. portions of distilled water in dialyzing tubing of ¼-in. diameter were dialyzed against 200 ml. of skimmilk and of washed milk (5% fat) for at least 24 hr. at 3° C. Radioactivity of the dialysates was determined.

RESULTS AND DISCUSSION

Added copper. Cu^{64} was added to mixed milk obtained from four cows that were on pasture. After adding the isotope, the milk was divided into two lots. Lot A was held 2 hr. at 37° C. before separation and fractionation. Lot B was given the temperature treatment used for activation of lipolysis or bodying of cream (cooled and stored for 2 hr. at 10° C., warmed to 30° C. for 10 min., re-

[5] H. O. Anger Co., Berkeley, California.

cooled and stored for 2 hr. at 10° C.), then warmed to 40° C., followed by separation and fractionation.

The results (Table 1) show that nearly all of the added copper was recovered in the skimmilk. Over 75% was associated with the redispersed casein. Only 2% of the total was associated with the washed fat globules. Calculations based

TABLE 1

Distribution of added Cu^{64} in milk

Sample description	Lot A Not previously cooled			Lot B Temperature activated	
	Activity	Activity of whole milk	Activity per 1% of protein	Activity	Activity of whole milk
	(c.p.m.[a])	*(%)*	*(c.p.m.)*	*(c.p.m.)*	*(%)*
Whole milk	8,720	100.0	2,430	9,190	100.0
Skimmilk	8,900	101.9	2,440	9,190	100.0
Centrifuged casein	6,600	75.6	2,200		
Centrifuged whey	1,410	16.2	2,320		
Cream	3,720	42.6		5,050	55.1
1st washed cream	1,540	17.6		1,680	18.3
2nd washed cream	1,500	17.2		1,520	16.5
3rd washed cream	1,540	17.6		1,430	15.5
4th washed cream	1,400	16.0		1,520	16.6
Membrane solution	1,470		3,230		
Melted fat	66				
1st wash water	1,490			1,430	
2nd wash water	198			226	
3rd wash water	68			93	
4th wash water	49			37	
Washed milk[b]	175	2.0		190	2.1

[a] Counts per minute, corrected for decay and background.
[b] Calculated on the basis of dilutions of 4th washed cream to 5% fat.

on the nitrogen content of the various fractions show that added copper is nonspecifically associated with all of the protein fractions considered, including the fat globule membrane proteins.

The activities of the various wash waters and washed creams indicate that the washing procedure did not remove excessive amounts of copper from the fat globules. The relatively high activity of the first wash water can be accounted for on the basis of skimmilk removed from the cream.

The temperature-activation or bodying treatment had no apparent effect on the distribution of added copper.

Natural copper. The distribution of natural Cu^{64} in milk from two cows is presented in Table 2. The cows were Jerseys, about 4 mo. in lactation and on pasture. In one experiment, only the first milk secreted after isotope infusion was examined (Cow 966). In the other experiment, the first three milks were examined (Cow 57).

Natural copper, unlike added copper, favored the fat phase of milk. From 9.7 to 21.2% of the natural copper was associated with the washed fat globules. Essentially all of the fat-globule copper was recovered in the churned membrane solution. The natural copper not associated with the fat globules was distributed between the redispersed casein and whey, approximately in proportion to protein

TABLE 2

Distribution of natural copper in milk from two cows

	Cow 966[a]			Cow 57[b]							
				First milking			Second milking			Third milking	
	Activity	Activity of whole milk	Activity per 1% of protein	Activity	Activity of whole milk	Activity per 1% of protein	Activity	Activity of whole milk	Activity per 1% of protein	Activity	Activity of whole mlik
	(c.p.m.)	*(%)*	*(c.p.m.)*	*(c.p.m.)*	*(%)*	*(c.p.m.)*	*(c.p.m.)*	*(%)*	*(c.p.m.)*	*(c.p.m.)*	*(%)*
Whole milk	6,540	100.0	1,830	12,400	100.0	3,470	1,660	100.0	462	1,020	100.0
Skimmilk	5,860	89.7	1,610	9,700	78.2	2,670	1,310	78.9	359	845	83.1
Isoelectric casein	4,570	70.0		7,060	57.0		1,120	67.8			
Isoelectric whey	1,080	16.5		1,770	14.2		206	12.4			
Centrifuged casein	4,780	73.2	1,570	7,710	62.2	2,570	966	58.4	318		
Centrifuged whey	1,030	15.7	1,680	1,370	11.0	2,240	270	16.3	443		
Washed milk[c]	634	9.7		2,630	21.2		285	17.2		173	17.0
Membrane solution	8,600		18,900	32,500		71,300	2,680		5,870		
Melted fat	134			254			55				

[a] 8.2 mc. infused.

[b] 25 mc. infused.

[c] Corrected to same fat content as whole milk.

concentration. The ratio of copper to protein was from 10 to 20 times greater in the membrane proteins than in the casein and the whey proteins.

There was a marked difference in copper distribution in the milks from these two cows. However, the over-all distribution in successive milks from the same cow did not differ greatly, even though total activity did. This is interpreted as indicating that the method of isotope administration yields results that are representative of natural copper distribution.

Comparison of natural and added copper. For a more direct comparison of the distribution of natural and added copper, both forms were studied in milk from the same cow. The cow was a Holstein, late in lactation, on dry feed, and producing milk that developed oxidized flavor spontaneously. Milk drawn just before isotope infusion was used for determining added-copper distribution; milk drawn 12 hr. after isotope infusion was examined for natural-copper distribution.

The results (Table 3) show that the distribution of Cu^{64} differed markedly in these two milks. Less than 3% of the added copper, but over 35% of the

TABLE 3

Distribution of natural and added Cu^{64} in a spontaneous milk

Sample description	Natural copper[a]			Added copper		
	Activity	Activity of whole milk	Activity per 1% of protein	Activity	Activity of whole milk	Activity per 1% of protein
	(c.p.m.)	*(%)*	*(c.p.m.)*	*(c.p.m.)*	*(%)*	*(c.p.m.)*
Whole milk	6,250	100.0	1,780	27,500	100.0	7,860
Skimmilk	3,840	61.4	1,070	29,000	105.2	8,050
Isoelectric casein	3,270	52.4	1,130	22,800	82.8	7,860
Isoelectric whey	1,050	16.8	1,310	4,740	17.4	6,780
Whey filtrate[c]	143	2.3		787	2.9	
4th washed cream[b]	2,230	35.7		771	2.8	
Membrane solution	25,700		51,400	8,250		16,500
Membrane filtrate	579[c]			38[d]		
Melted fat	326			135		
Membrane from melted fat	33,300					
Membrane lipids	1,960			119		
Membrane proteins	23,000		51,000	6,760		15,000

[a] 56 mc. infused.
[b] Diluted to same fat content as whole milk before counting.
[c] Saturated with ammonium sulfate to remove proteins.
[d] Centrifuged at pH 4.8 to remove proteins.

natural copper, was associated with the washed fat globules. When the membrane material was fractionated with ethanol-ether, essentially all of the activity was recovered in the redispersed membrane proteins (for both added and natural copper). The amount of natural copper per 1% of protein was nearly 50 times greater in the membrane proteins than in the casein and whey proteins. For added copper this ratio was only two to three times greater than in the skimmilk proteins.

The activity remaining in the filtrate after saturating the whey with ammonium sulfate may have resulted from incomplete protein precipitation or

from splitting the copper-protein bond. As shown later, it was not attributable to unbound copper.

Added iron. In an experiment on the distribution of added Fe^{59}, the milk was divided into two lots; one lot was held 4 hr. at 37° C. before fractionation and the other 36 hr. at 3° C.

The results (Table 4) show that essentially all of the added iron was associated with the skimmilk. The washed creams had only negligible activities.

TABLE 4

Distribution of added Fe^{59} in milk

Sample description	Aged 4 hr. 37° C.		Aged 48 hr. 3° C.	
	Activity	Activity of whole milk	Activity	Activity of whole milk
	(c.p.m.)	*(%)*	*(c.p.m.)*	*(%)*
Whole milk	3,530	100.0	3,350	100.0
Skimmilk	3,490	98.8	3,470	103.6
Centrifuged casein	3,210	90.8	3,670	109.6
Centrifuged whey	799	22.6	690	20.6
Isoelectric whey	1,030	29.1		
Cream[a]	2,220	8.6	2,100	8.5
1st washed cream	145	0.6	187	0.8
2nd washed cream	26	Negligible	16	Negligible
3rd washed cream	5	Negligible	19	Negligible
4th washed cream	8	Negligible	30	Negligible

[a] Activity for cream is corrected to the same fat content as the milk.

Although nitrogen determinations were not included in this experiment, the distribution of added iron appeared to be about proportional to the expected concentration of the skimmilk proteins. The redispersed casein accounted for most of the activity in the original milk. There was no obvious difference related to the two storage treatments.

Iron is generally considered to be much less effective than copper as a catalyst for oxidized flavor. These results show that iron has less affinity for the fat globule surface than does copper, since 2–3% of the added copper was associated with the fat globules.

Natural iron. The distribution of natural Fe^{59} in milk from Cow 57 is presented in Table 5. Natural iron, like natural copper, was highly concentrated at the fat globule membrane. Natural iron not associated with the fat globules was distributed between the casein and whey proteins. Unlike natural copper, natural iron favored the whey proteins over the casein.

The radioactivity in the second and eighth milks differed considerably from the first milk. This may be the result of a slower metabolic turnover rate for iron than for copper. In the case of copper, the first milk after isotope infusion consistently contained the most activity, whereas with iron the second milk had the maximum activity (Table 6), and the fifth milk still had more activity than the first milk. After 30 days, the cow (and the milk) still retained a level of radioactivity significantly above background.

Dialyzability of natural and added copper and iron. Differences in dialyzing characteristics of natural and added metals provided information about the

TABLE 5

Distribution of natural Fe^{59} in milk[a]

Sample description	First milking		Second milking		Eighth milking	
	Activity	Activity of whole milk	Activity	Activity of whole milk	Activity	Activity of whole milk
	(c.p.m.)	*(%)*	*(c.p.m.)*	*(%)*	*(c.p.m.)*	*(%)*
Whole milk	1,240	100.0	2,020	100.0	687	100.0
Skimmilk	1,020	82.4	1,060	52.8	404	58.8
Centrifuged casein	595	47.9	423	20.9	127	18.5
Centrifuged whey	341	27.5	460	22.7	174	25.3
Isoelectric whey	456	36.7	588	29.1	225	32.7
Cream[b]	1,960	37.9	4,860	57.7	1,930	42.8
1 st washed cream	1,230	23.9	3,920	46.5	1,720	38.1
2nd washed cream	1,090	21.1	4,000	47.4	1,680	37.1
3rd washed cream	1,030	20.1	2,860	33.9	1,540	34.2
4th washed cream	1,060	20.7	3,120	37.1	1,450	32.2
Membrane solution	1,300		2,510		2,830	
Melted fat	146		345		87	

[a] 9.5 mc. infused.
[b] Activity for cream is corrected to same fat content as the milk.

TABLE 6

Activity of natural Fe^{59} in successive milks after isotope infusion

Milking after infusion	Time of secretion	Activity
	(days)	*(c.p.m.)*
1st	0	1,240
2nd	0.5	2,020
3rd	1.0	1,820
4th	1.5	1,340
5th	2.0	1,400
6th	2.5	1,090
7th	3.0	787
8th	3.5	687

TABLE 7

Influence of pH on dialysis of natural and added Cu^{64} and Fe^{59} in milk

Sample description	Cu^{64}			Fe^{59}		
	pH	Natural	Added	pH	Natural	Added
		(c.p.m.)	*(c.p.m.)*		*(c.p.m.)*	*(c.p.m.)*
Skimmilk	6.7	1,360	21,200	6.8	369	3,280
Skimmilk dialysate	6.7	16	345	6.8	0	122
Skimmilk dialysate	5.0	0	966	6.0	0	129
Skimmilk dialysate	4.0	4	1,300	5.5	0	129
Skimmilk dialysate	3.0	6	6,700	5.0	0	149
Skimmilk dialysate				4.5		279
Skimmilk dialysate				4.0		498
Washed milk	6.7	792	590	6.7	399	0
Washed milk dialysate	6.7	1	4	6.7	0	0
Washed milk dialysate	5.0	1	6	4.5	0	0
Washed milk dialysate	4.0	1	8			
Washed milk dialysate	3.0	0	15			

stability of the metal-protein bond. Results of some experiments in which small volumes of distilled water were dialyzed against much larger volumes of skimmilk or washed milk containing an isotope are summarized in Table 7. Natural copper in skimmilk and in washed milk was not dialyzable, and the association of natural copper was not affected by a decrease in pH from 6.7 to 3.0. Added copper associated with the fat globule likewise was not dialyzable over this pH range. However, added copper in skimmilk was slightly dialyzable at pH 6.7, and the amount of unbound copper increased with a decrease in pH.

Results for iron are similar to those for copper. Natural iron was not dialyzable over the pH range studied. Added iron was slightly dialyzable at the pH of milk, and the amount of unbound iron increased as the pH was decreased.

For copper that is not associated with the fat globule membrane, the difference in dialyzing characteristics between natural and added copper indicates a different chemical association for these two sources of copper. For copper associated with the fat globule membrane, the similarity of dialyzability of natural and added copper suggests a similar chemical association. Thus, natural and added copper associated with the fat globule membrane could have similar properties in the catalysis of oxidized flavor. The observation that no added iron was associated with the fat globule membrane could partially explain the lower catalytic activity of added iron as compared to added copper.

Data presented previously demonstrated a relation between the concentration of natural copper and the incidence of spontaneous oxidized flavor in milk produced by cows on an alfalfa hay–grain concentrate ration. Results discussed above and some presented elsewhere (6) suggest that the distribution of natural copper is also a factor in determining the susceptibility of milk to oxidized flavor.

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THE COCONUT-LIKE FLAVOR DEFECT FROM MILK FAT. III. OBSERVATIONS ON THE ORIGIN OF δ-DECALACTONE IN FAT-CONTAINING DAIRY PRODUCTS [1,2]

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SUMMARY

Evidence, experimental and theoretical, is presented which tends to indicate that the precursor of the coconut-like flavor compound, δ-decalactone, in fat-containing dairy products is 5-hydroxydecanoic acid. This compound is postulated to exist in native butterfat as a simple ester, uncharacterized. By low-temperature crystallization of butterfat from acetone, the ester in question was observed to concentrate in the low-melting fraction (−50° C. filtrate). It was further resolved into a fraction representing about 0.4% of the original fat by adsorption chromatography on alumina.

Early in the storage life of certain fat-containing dairy products, a coconut-like flavor defect is noticeable. Mussett *et al.* (6) have presented evidence that this off-flavor is associated with the lipid phase, and that heat treatment and storage are of primary importance in its development. The compound responsible for the coconut-like flavor has been isolated and identified as δ-decalactone by Keeney and Patton (5). They also demonstrated the presence of this compound in dried whole milk, dried cream, and evaporated milk. The purpose of this paper is to report investigations on origin and mode of formation of the flavor compound.

EXPERIMENTAL PROCEDURE

Preparation of the butteroil. Fresh, raw cream obtained from the University Creamery was churned. The resulting butter was washed three times with cold water (10° C.). Each washing was equivalent to the original volume of the cream. The butter then was melted and washed four times with warm water (45° C.). The aqueous phase was separated in a small DeLaval cream separator from the butteroil after each washing. The temperature of 45° C. was never exceeded during any part of the butteroil preparation.

Low-temperature crystallization of butteroil from acetone. Mussett *et al.* (6) obtained a high-melting fraction by submitting partially crystallized butteroil to hydraulic expression. This fraction exhibited less tendency to develop the

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coconut-like flavor. This observation appeared to be an excellent starting point for the concentration of the precursor of δ-decalactone. However, for more clear-cut fractionation, it was decided to utilize low-temperature crystallization of butteroil from acetone rather than the hydraulic expression method. Butteroil was fractionated by low-temperature crystallization as shown in Figure 1. Characteristics of the fractions are summarized in Table 1.[4]

TABLE 1

Characteristics of fractions obtained by low-temperature crystallization of butteroil from acetone[a]

Fraction	Appearance	Weight	Iodine No.	NH_2OH test (2)	Coconut-like odor[b]
	(at 22° C.)	*(g.)*			
−22° C. ppt. (A-1)	Solid	52	3	−	−
−50° C. ppt. (A-2)	Liquid	13	64	+	−
−70° C. ppt. (A-3)	Liquid	5	70	++	+++
−70° C. filtrate (A-4)	Liquid	2	101	+++	++++

[a] Starting with 100 g. of fat in 500 ml. of acetone.
[b] After autoclaving for 30 min. at 120° C. in sealed container.

The −22° C. precipitate was largely saturated glyceride material (I.V. 3). The −50 and −70° C. precipitates had approximately the same degree of unsaturation (I.V. 64 and 70, respectively), whereas the −70° C. filtrate was considerably more unsaturated (I.V. 101). The hydroxylamine reaction (2) was performed on the various fractions and was considered positive when a purple color was evident in the reaction mixture. In general, the short-chain acid esters, including δ-decalactone, give a soluble purple pigment in this test; whereas, long-chain esters (C_{12} and higher) give a reddish, insoluble pigment.

Practically all, if not all, of the coconut-like flavor potential appeared to be concentrated in the −50° C. filtrate. Since it was difficult to maintain a temperature of −70° C., and since most of the glycerides were removed at −22 and −50° C., it appeared that very little was gained in working below −50° C.

Hydrogenation studies. The possibility that the lactone may arise from the hydration or rearrangement of a decenoic acid has never been proven or disproven to any satisfaction. The fact that 9-decenoic acid has been eliminated as a precursor (4) does not necessarily eliminate other possible decenoic acids or the decomposition of an unsaturated acid. In the original study (6), the butteroil was hydrogenated using commercial methods, and the resulting fat was not observed to produce the typical dry whole milk flavor. In commercial practice, the fat is alkali-washed and deodorized before hydrogenation. There is a great possibility that either or both of these treatments could have rendered the butteroil free of its lactone-producing potential.

A preliminary experiment to verify the effect of hydrogenation on the lactone-producing capacity of the butteroil was attempted. Freshly prepared butteroil was hydrogenated as follows: 300 g. of butteroil was dissolved in 700 ml. of

[4] In the particular experiment for which data are given, manipulative losses were higher than usual.

sulfur-free hexane. This solution was placed in a Parr (Series 3910) laboratory hydrogenation apparatus and 4 g. of spongy nickel catalyst was added. Hydrogenation was effected at 21 to 25° C. and 30 to 50 lb. hydrogen presure. When hydrogen uptake had ceased (approximately 36 hr.), the reaction mixture was filtered to remove the catalyst and then evaporated under reduced pressure at 10° C. to eliminate solvent. The iodine number of the resulting butteroil was reduced from 34 to <1. Samples of both the fresh and hydrogenated butteroil were autoclaved to give an indication of lactone potential. Both samples, hydrogeated and fresh butteroil, produced approximately the same intensity of lactone odor.

In a further investigation, the −50° C. filtrate fraction was prepared from fresh butteroil according to Figure 1. A portion of the fraction was hydrogenated by the method outlined above and part was retained as a control, this latter

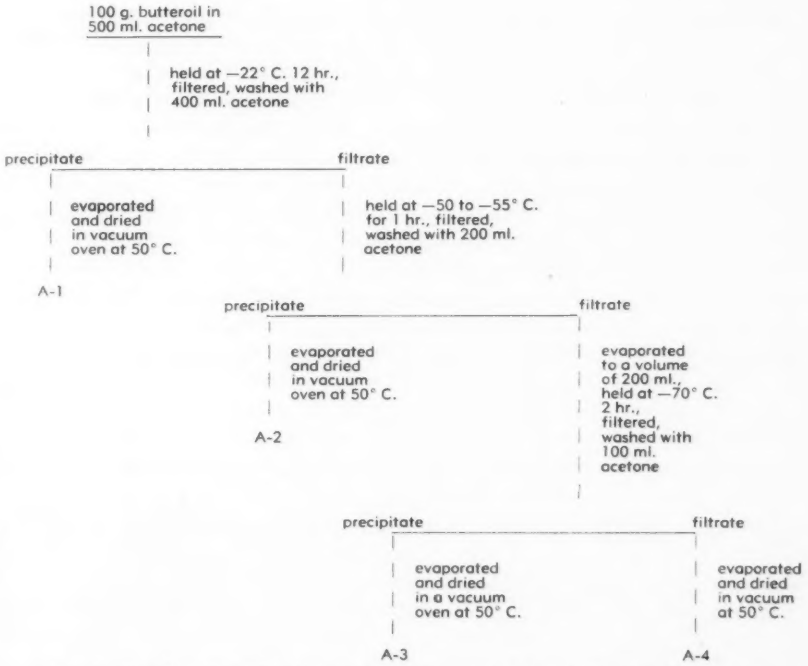

100 g. butteroil in 500 ml. acetone
held at −22° C. 12 hr., filtered, washed with 400 ml. acetone
precipitate
filtrate
evaporated and dried in vacuum oven at 50° C.
held at −50 to −55° C. for 1 hr., filtered, washed with 200 ml. acetone
A-1
precipitate
filtrate
evaporated and dried in vacuum oven at 50° C.
evaporated to a volume of 200 ml., held at −70° C. 2 hr., filtered, washed with 100 ml. acetone
A-2
precipitate
filtrate
evaporated and dried in a vacuum oven at 50° C.
evaporated and dried in vacuum at 50° C.
A-3
A-4

FIG. 1. Fractionation of butteroil by low-temperature crystallization from acetone.

fraction being designated B-1. The hydrogenation process reduced the iodine number from 103 to <1. The hydrogenated material was again submitted to the low-temperature crystallization procedure to obtain a −50° C. filtrate fraction. This crystallization was performed using a ratio of 1 g. of fat to 5 ml. of acetone. The filtrate at −50° C. was evaporated under reduced pressure at 10° C. and the residue was designated Fraction B-2. Both Fraction B-1 and Fraction

B-2 developed a strong odor of δ-decalactone when autoclaved as previously described; however, they were essentially odorless before such treatment. The presence of δ-decalactone in the autoclaved samples was confirmed by extracting the samples with 5% aqueous Na_2CO_3, removing nonextractable material with ether, acidifying this extract to pH 2 to regenerate the lactone, and demonstrating the lactone paper-chromatographically as its hydroxamate by the method of Keeney and Patton (5). Spots which had moved in coincidence with the hydroxamate of authentic δ-decalactone were clearly observed. Application of this procedure to unheated portions of Fractions B-1 and B-2 yielded no odor or paper-chromatographic evidence of the lactone. The results of these experiments concerning hydrogenation are considered rather strong evidence against an unsaturated acid or an unsaturated triglyceride as an origin of δ-decalactone.

Alumina chromatography of the low-melting fraction. Disposition of the lactone precursor in the low-melting fraction (−50° C. filtrate) suggested that further concentration of the precursor might be effected by adsorption chromatography of the fraction on alumina. Neutral alumina was prepared by the method of Reichstein and Shoppee (8). It was kept activated by heating in an oven at 100° C. for a period of 12 hr. A 15- by 200-mm. glass chromatographic column was used to support the absorbant. The alumina (30 g.) was slurried in petroleum ether and poured into the column in small portions.

After each addition of alumina, a few milliliters of petroleum ether were added to the column. Keeping a plentiful supply of solvent in the column during the addition of the adsorbant resulted in an evenly packed, tight column. The low-melting fraction, 14 g. from 200 g. of fresh butteroil, was dissolved in 25 ml. of petroleum ether and applied to the column. A series of solvents in 50-ml. volumes was applied in order of increasing eluting power, according to Trappe (10). These ranged from petroleum ether to acetone (see Table 2). Each

TABLE 2

Fractionation of the low-melting fraction[a] from butteroil by alumina chromatography in determination of the origin of δ-decalactone

Fraction	Eluting solvent	Weight of fraction	Total fraction	Original fat	Lactone odor
		(g.)	*(%)*	*(%)*	
F-1	Petroleum ether	9.8	70	4.9	−
F-2	Carbon tetrachloride	0.92	6.0	0.46	−
F-3	Toluene	0.25	2.0	0.13	−
F-4	Benzene	0.22	1.4	0.11	−
F-5	Chloroform	0.81	5.7	0.41	++++
F-6	Ethyl ether	0.24	1.4	0.12	−
F-7	Ethyl acetate	0.02	0.14	0.01	−
F-8	Acetone	0.05	0.28	0.02	−
Total		12.3	86.9	6.16	

[a] Obtained in the −50° C. filtrate from crystallization of butteroil from acetone.

solvent system was collected as a fraction from the column. The odor of the solvent as it came from the column was used to determine the end of one fraction and the beginning of the next. The fractions were evaporated under reduced

pressure and the residue for each was weighed. The capacity of the fractions to generate δ-decalactone was determined by autoclaving (120° C. — 30 min.) the individual residues in sealed containers and then noting the odor of each. Two experiments were performed; since the results are very similar, the data of only the more detailed one are presented (Table 2).

The fraction eluted by chloroform (F-5) was the only one which generated a strong, clearly evident odor of δ-decalactone on heating. This same fraction also gave a strong Liebermann-Burchard test for cholesterol (3); whereas, Fraction F-6 was decidedly less positive and Fraction F-4 was negative in this test. The data in Table 2 show that the fraction which contained the lactone precursor amounted to 5.7% of the low-melting fraction, or approximately 0.4% of the original butteroil.

Effect of sodium carbonate washing. During preliminary experiments on the recovery of δ-decalactone from fresh butteroil, using a sodium carbonate wash, an odor resembling the lower fatty acids was encountered when a 10% solution of sodium carbonate was used. Keeney and Patton (4) reported that a certain amount of moisture was required for maximum production of the lactone odor by autoclaving butteroil. In view of these observations, an hypothesis was advanced that the mechanism which yields lactone from precursor is a hydrolytic type reaction. In a study of this point, freshly prepared butteroil (300 g.) was added to each of four 1,000-ml. vacuum flasks. Each flask was assigned an equal volume (330 ml.) of 0, 1, 5, and 10% aqueous sodium carbonate solution, respectively. The flasks were stoppered, evacuated by means of a water aspirator, sealed, and shaken on a mechanical shaker for 4 hr. The vacuum then was released and any lactone [5] was separated from the fat by acidifying the extract to pH 2 with 5% aqueous hydrochloric acid, extracting successively with four 330-ml. volumes of ethyl ether, combining the ether extracts, and evaporating them to dryness.

The odor of each of the residues was noted. No odor resembling δ-decalactone was detectable in the distilled water or the 1% washing; a questionable odor, which could be classed as lactone, was evident in the 5% washing; the 10% washing presented a strong rancid odor, which could have masked the odor of any lactone that might be present. The hydroxamate derivatives were prepared from the residues and chromatographed as previously described. The 10% sodium carbonate extract yielded a spot corresponding to the authentic δ-decalactone. No other extract gave a similar spot. The fact that δ-decalactone was recovered by extraction with 10% sodium carbonate solution did not necessarily confirm a hydrolysis reaction. Rearrangement or condensation of some substance or substances to form the lactone during extraction would not be precluded. Milder conditions of hydrolysis would be desirable to evaluate further these possibilities.

Enzymatic hydrolysis. Enzymatic hydrolysis of butteroil was considered to be the mildest hydrolytic treatment available. A commercial lipase (Nutritional

[5] It is presumed that any δ-decalactone which is liberated from the fat would exist at this point as the sodium salt of 5-hydroxydecanoic acid.

Biochemicals Corp. steapsin) was employed. Fresh butteroil (300 g.) was added to each of three 1,000-ml. vacuum flasks. Eahc flask received an equal volume (330 ml.) of one of the following solutions: distilled water; 0.1% solution of lipase heated to 100° C. for 15 min.; 0.1% solution of lipase (unheated). Evacuation and shaking of the mixtures were performed in exactly the same manner as followed in the sodium carbonate washing. The method of extracting the δ-decalactone was changed in this study. In place of the sodium carbonate extraction previously used, the butteroil was exhaustively extracted with warm water (50° C.). The extracting water was then cooled to room temperature and extracted with 10- to 100-ml. portions of ethyl ether. The ether was dried over anhydrous sodium sulfate, filtered, and evaporated. A slight fatty-like residue was obtained in the case of the distilled water and heated lipase solution, but no δ-decalactone odor was apparent. The unheated lipase solution yielded a large amount of white semisolid residue. The residue had the same rank odor as the 10% sodium carbonate extraction, but of a greater intensity. The residues were divided into two equal portions and the hydroxamate derivative of each was prepared and chromatographed by the method previously described (5). All three samples produced a purple spot in the region usually occupied by butyrate; however, only the residue from the sample treated with unheated lipase produced a spot corresponding to the authentic δ-decalactone.

The purple spot produced by butyrate sometimes may be confused with that of δ-decalactone. The remaining portions of the residues were shaken with 50 ml. of a 5% aqueous sodium bicarbonate solution for 3 hr., to selectively remove free acids. The samples then were extracted with ethyl ether. The ether was evaporated and the samples then shaken with 50 ml. of a 5% aqueous solution of sodium carbonate for 3 hr. and the lactone extracted in the manner previously described. No definite odor was observed in any of the residues except that derived from the sample treated with active lipase. This latter residue had a strong odor of δ-decalactone. It was taken up in 0.1 to 0.2 ml. of carbon tetrachloride and analyzed in a Perkin-Elmer Model 21 Infrared Spectrophotometer. The spectrum (3 to 15 μ) coincided in all respects with that observed previously (4) for δ-decalactone. The hydroxamates of the residues were prepared and paper-chromatographed. The unheated lipase sample was the only sample exhibiting a spot. This spot coincided with that from the hydroxamate of authentic δ-decalactone.

DISCUSSION

Exploratory investigations were conducted with the object of determining the origin of δ-decalactone in fat-containing dairy products and of postulating a possible mechanism for the formation of this lactone. Investigators (7) have proposed the mechanism of rearrangement of a double bond in a decenoic acid to the delta position, followed by lactonization. These workers later presented evidence that eliminated 9-decenoic acid as a possible precursor (4). If the mechanism outlined were involved in formation of the flavor compound, then hydrogenation of the butteroil should prevent or reduce the lactone potential of the butteroil. The present study revealed no apparent decrease in flavor potential between untreated and hydrogenated butteroil.

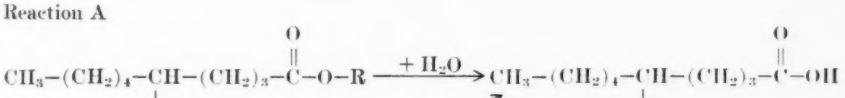
The fact that the δ-decalactone precursor was not removed by the low-temperature crystallization of the hydrogenated −50° C. filtrate fraction of butteroil, would tend to indicate that the precursor is present in a minor fraction of the fat. If the bonding were in the form of a glyceride, one would expect some random distribution of the precursor throughout the fat. Even if a glyceride precursor were highly unsaturated, the law of probability would have it appearing in a higher-melting fraction from the low-temperature crystallization of the hydrogenated −50° C. filtrate. Therefore, it is believed that the precursor is rather simple and homogeneous in composition.

The literature (1, 9) describing the formation of lactones by rearrangement and hydration of double bonds shows that the gamma rather than the delta isomer is formed. To form the delta-lactone, an easily hydrolyzable or hydrated group, such as a halogen or the hydroxyl group itself, must be present in the delta position. Thus, in all probability the hydroxyl group of the lactone from milk fat is fixed in the delta position within the precursor.

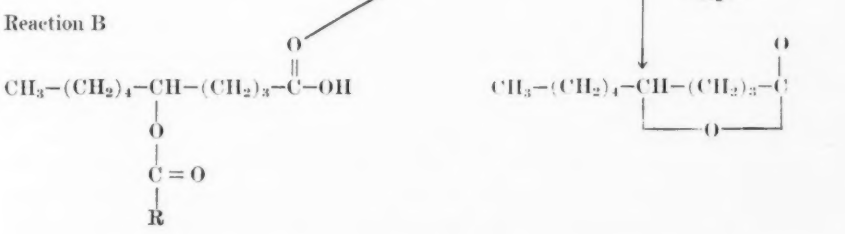
The production of δ-decalactone, resulting from the hydrolysis of butteroil by sodium carbonate or lipase, seems to establish the mechanism for such a reaction as the hydrolysis of an ester. δ-Decalactone was shown to be present in both cases by paper chromatography, and in the study of enzymatic hydrolysis, the identity of the compound was confirmed by infrared spectra.

The information obtained to date indicates that δ-decalactone is derived by a hydrolysis which liberates 5-hydroxydecanoic acid. The following equations represent possible reactions whereby δ-decalactone would be formed in products containing butterfat:

Reaction A



Reaction B



Reactions A and B show lactone precursors in which either the carboxyl or hydroxyl group of 5-hydroxydecanoic acid is esterified. A third type of precursor in which both groups are bound is yet another alternative. None of the indicated precursors is clearly precluded by the data thus far. However, the ease with which δ-decalactone forms in milk products places in question a precursor

in which both groups are bound. Chemical characterization of the precursor of δ-decalactone is the subject of continuing research at this laboratory.

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TAXONOMIC STUDY OF HIGH CARBON DIOXIDE–PRODUCING LACTIC ACID STREPTOCOCCI ISOLATED FROM MIXED-STRAIN STARTER CULTURES [1]

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SUMMARY

A taxonomic and serological investigation was carried out on strains of high CO_2-producing lactic acid streptococci isolated from commercial mixed-strain starter cultures causing the floating curd defect in cottage cheese. Non-CO_2-producing lactic acid streptococci found in mixed-strain starter cultures also were studied. Known cultures designated as *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetilactis*, *Leuconostoc citrovorum*, and *Leuonostoc dextranicum* were included for comparative purposes.

The high CO_2-producing lactic acid streptococci were identified in this study as *S. diacetilactis*. *S. lactis* and *S. cremoris* species easily were identified by salt tolerance, growth temperature and hydrolysis of arginine. *S. diacetilactis* gave characteristic cultural and serological reactions and, along with *S. lactis* and *S. cremoris* organisms, was distinguishable from *Leuconostoc* species by litmus milk reaction at 30° C.

The name *Streptococcus cremoris* var. *aromaticus* was suggested to identify two cultures which seemed intermediate between *S. cremoris* and *S. diacetilactis*. Test methods employed did not distinguish between organisms designated as *L. citrovorum* and *L. dextranicum*, but the one culture of *Leuconostoc mesenteroides* used in the study was easily identified by its ability to liberate ammonia from arginine which none of the associative organisms could do.

Previous investigations (16, 17, 18) have shown that the floating curd defect encountered in the manufacture of cottage cheese is related in most instances to the presence in the mixed-strain starter cultures of lactic acid streptococci capable of producing large volumes of CO_2. Mixed-strain cultures, from which the higher carbon dioxide–producing lactic streptococci were eliminated, uniformly produced normal cheese with no tendency toward the floating curd defect. Such cultures suitable for cottage cheese could be prepared by combining homofermentative, low gas-producing lactic streptococci and compatible *Leuconostoc* species.

Single-strain cultures isolated from the high gas-producing, mixed-strain cultures were divided generally into four different groups on the basis of activity (rate of acid production) and CO_2 production. These were high acid–high gas, low acid–high gas, high acid–low gas, and low acid–low gas.

The taxonomy of these four groups of streptococci and, in particular, the two gaseous types was in question. Therefore, an investigation was undertaken to determine how each type might be classified on the basis of cultural, biochemical, and serological criteria for the lactic acid streptococci as established by Sherman (20), Briggs (2), Briggs and Newland (3), Mattick and Shattock (11), and other workers. The results of the investigation are presented in this paper.

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Krishnaswamy and Babel (9) and Swartling (22) have reviewed earlier reports on CO_2 and flavor production by lactic acid streptococci. Sherman (21) also commented on the possible classification of various flavor and CO_2-producing types of lactic streptococci.

EXPERIMENTAL PROCEDURE

Known species of representative organisms that might be used in lactic streptococcus starter cultures were obtained from commercial cultures and various laboratories throughout the United States, as well as one in Europe. The species included organisms designated as *S. lactis, S. cremoris, S. diacetilactis, L. citrovorum,* and *L. dextranicum.* One culture of *L. mesenteroides* (P-60) also was included for comparative purposes, although this organism is not used in controlled dairy fermentations. Single-strain cultures were isolated from ten mixed-strain starter cultures, as well as from a sample of floating curd cottage cheese by serial dilution plating, using the "lactic agar" developed for lactic acid bacteria (4). A total of 189 single-strain cultures was studied. Among these, 141 were isolated from commercial mixed-strain starter cultures.

Each organism used in this study was examined for at least 34 different cultural reactions. The tests were carried out either in duplicate or, when the number of tubes made this difficult, experiments were repeated. Precipitin tests were run on known cultures of *S. diacetilactis* and *Leuconostoc* organisms. They also were run on organisms believed to be *S. diacetilactis* isolated from mixed-strain starter cultures. The following procedures were used:

Cell morphology and cultural reaction in litmus milk. Methylene blue stains of litmus milk cultures incubated at 21° C. were prepared and examined for cell size, shape, and arrangement. Incubation of tubes not showing rapid acid coagulation was continued until such a reaction occurred or for a 30-day period. Cultures producing no visible reaction in the litmus milk after 30 days were examined again for viability by a second methylene blue stain.

Catalase production. Three per cent hydrogen peroxide (1 to 2 ml.) was added to 24-hr. "lactic broth" (4) cultures of each organism. Observations were made for gas evolution.

Culture activity and acid coagulation of milk. The rate of acid production of each culture in sterile 10% reconstituted nonfat milk was measured by titrating the acid produced from a 5% inoculum in 5 hr. at 30° C. with standard alkali dispensed by a Cannon electrotitrimeter. Results were expressed as per cent lactic acid. Cultures similarly inoculated and incubated for 48 hr. at 30° C. were observed for ability to coagulate milk.

Hydrolysis of arginine. The method of Niven *et al.* (13) was used.

Survival at 60° C. for 30 min. One drop of inoculum from a 24-hr. lactic broth culture was added aseptically to 10 ml. of fresh sterile broth, which was heated at 60° C. for 30 min. At the end of the time period, the tubes were withdrawn and immediately placed in a second water bath at 30° C. They were incubated for 48 to 72 hr. and observed for growth.

Growth at 10, 40, and 45° C. One drop of inoculum from a 24-hr. lactic broth

culture was added aseptically to 10 ml. of fresh sterile broth. The tubes were placed in water baths maintained at the desired temperatures and cultures observed for growth after 48 hr., with the exception of those at 10° C., which were read at the end of a 2-wk. period.

Growth at pH 9.2 and 9.6. Sterile N/1 sodium hydroxide was added aseptically to 10-ml. volumes of lactic broth so that pH values of 9.2 and 9.6 were obtained (about 0.2 and 0.3 ml., respectively, were required). The resulting media were inoculated as described in tests on growth temperatures, incubated 48 hr. at 30° C., and observed for growth.

Growth in presence of 4.0 and 6.5% sodium chloride. The sodium chloride concentration of lactic broth was increased to 4.0 and 6.5% in two different lots of media. Tubes were inoculated with each culture as described in tests on growth temperatures, incubated at 30° C. for 48 hr. and observed for growth.

Production of aroma compounds in milk. The method of King (8) was used. Organoleptic observations also were made.

Associative production of aroma compounds. Each *Leuconostoc* organism was combined with at least one rapid acid-producing strain of *S. lactis* and also *S. cremoris* in separate experiments, and tested for production of flavor and aroma compounds organoleptically and by the method of King (8). Cultures used were *Leuconostoc* species obtained from various laboratories, as well as strains isolated from mixed-strain commercial starter cultures. They were tentatively classified as members of the *Leuconoctoc* genus because of their inability to produce acid in litmus milk upon extended incubation at 21 and 30° C. The associative plus lactic streptococcus mixtures were prepared by adding 1 ml. of a 48-hr. culture of the former and 0.1 ml. of a 24-hr. milk culture of the latter to 10 ml. of sterile 10% reconstituted nonfat milk solids. The cultures were incubated at 21° C. for from 18 to 20 hr. and tested as indicated. Although it might have been desirable in some instances to carry such cultures through several subcultures, results in general provided desired information regarding products of associative growth of these organisms. Control tests also were run on each single-strain culture used in the combinations.

Gas production in milk culture. The manometric method described in an earlier paper (16) was used to measure the amount of CO_2 produced by each strain in milk culture.

Utilization of citrate. A modified method of Gibson and Abdel-Malek (5) was used. Each culture was tested in three different media simultaneously. Medium No. 1 contained nutrient gelatin (Difco), 0.25% yeast extract, 0.5% glucose, 10.0% filtered tomato juice, 0.01% brom cresol purple, and 3.0% citric acid–potassium citrate mixture to buffer the medium at pH 5.5 to 6.0. Medium No. 2 was the same as Medium No. 1, except that no glucose was added. Medium No. 3 was the same as Medium No. 1, except no citric acid–potassium citrate mixture was added and the pH was 6.8 to 7.0.

To make the test, the media were liquefied by immersion of the tubes in a 45° C. water bath and diluted from double to single strength by the addition of

the appropriate filter-sterilized substrate, also tempered to 45° C. Three drops of an 18-hr. lactic broth culture (a 48-hr. culture was used in case of the slower-growing *Leuconostoc* species) of each organism was added to each of the three types of media. After thorough mixing in a careful manner to avoid bubbles, the tubes were chilled to allow gel formation. Melted nutrient agar at 45° C. then was poured gently into the tubes to provide a 2- to 3-cm. agar layer over the medium. The tubes were incubated at 30° C. and observed for acid and gas production.

Carbohydrate utilization. Two-milliliter quantities of lactic broth medium without carbohydrate and containing 0.02% brom cresol purple were dispensed into 12 x 120 mm. tubes and autoclaved at 15 lb. pressure (121° C.) for 10 min. After cooling, 2 ml. of 1% filter-sterilized carbohydrate was added aseptically to each tube to provide a single-strength test medium containing 0.50% carbohydrate. The test media were inoculated in duplicate with a loop of inoculum (0.01 ml.) from a 24-hr. lactic broth culture and incubated at 30° C. for 48 hr. Slow-growing cultures such as *Leuconostoc*, which showed no evidence of growth in 48 hr., were incubated five days longer at room temperature (25° C.).

Serological tests. *S. lactis* E and *S. lactis* 7962 were selected for immunizing rabbits to prepare Group N anti-sera. Washed cells from 18-hr. lactic broth cultures were diluted to a predetermined volume in sterile physiological saline, and 1-ml. amounts injected every other day into the marginal ear vein, for a total of 12 injections. The antibody-containing serum was prepared from 20-ml. ear-vein bleedings into sterile centrifuge tubes. After the clot was formed, the blood was centrifuged and the clear serum used in the precipitin tests. The method of Lancefield (10) was used for preparation of cell extracts. The procedure described by Shattock (19) was followed for precipitin tests.

RESULTS

Characteristics common to all cultures. The cells from each culture were coccoid, 0.5 to 1.0 μ in diameter, occurring singly, in pairs and/or in short and/or long chains. No apparent correlation was evident between cell morphology or arrangement and any other property except, perhaps, in the case of *Leuconostoc* species isolated from mixed-strain cultures. Twenty-four-hour cultures of these strains showed little affinity for methylene blue, since the cells in stained preparations were difficult to see. This was not observed when reference *Leuconostoc* organisms were stained with methylene blue.

None of the organisms studied was capable of growing in the lactic broth base used for sugar utilization studies, unless a carbohydrate was present.

Morphology, cell arrangement, and the absence of catalase indicated that all cultures studied were members of the *Streptococcus* or *Leuconostoc* genus.

Results for known species. The results of the cultural and biochemical tests for each known organism appear in Table 1. Only those tests considered important from a taxonomic standpoint appear in the table. Several of the strains presumed to be *Leuconostoc* do not have species designations, since they were received from other sources without species identification.

Results for isolated cultures. The results of the cultural and biochemical tests performed on isolated cultures also appear in Table 1. When two or more strains isolated from a mixed culture showed identical test reactions, one was selected for reporting purposes.

General results of cultural and biochemical tests. Some correlation was evident between results of the activity and milk coagulation tests. However, a few known organisms showing slow rate of acid formation by the activity test continued producing acid so that the milk was ultimately coagulated within 24 hr. at 30° C. This appears to be another reflection of the difference between slow and fast strains. With one exception, none of the *Leuconostoc* strains produced sufficient acid to coagulate the milk in 48 hr. at 30° C., while all of the *S. lactis, S. cremoris,* and *S. diacetilactis* strains were able to accomplish this. The one exception (*L. citrovorum* 8082) was atypical in other respects and considered improperly classified; it grew at 10 and 40° C., hydrolyzed arginine, and produced a rennet-type curd which was completely proteolyzed in about 1 wk. at room temperature. This is typical of *Streptococcus liquefaciens* of the enterococcus group, and Strain 8082 may be representative of this species. Thus, genus identification of all known *Leuconostoc* strains used in this study was accomplished by incubation in litmus milk at 30° C. The *Leuconostoc* species isolated from mixed-strain starter cultures also failed to cause acid coagulation of litmus milk in 48 hr. at 30° C.

It was noted that only a few strains (30 of 189) were not able to survive 30 min. at 60° C. Growth of surviving cells was not evident in most instances for from 48 to 72 hr., which indicated that small numbers of cells were surviving. This phenomenon also has been observed by Abdel-Malek and Gibson (1) for *S. lactis* cells held at 60° C. for 30 min.

None of the *Leuconostoc, S. lactis,* or *S. cremoris* organisms appeared to be capable of producing aroma compounds in single strains when tested chemically and organoleptically. However, most of the *Leuconostoc* were capable of producing them in combination with a homofermentative lactic streptococcus. Furthermore, flavor and aroma were noticeable in several of the combinations that gave a negative King test, which indicated a greater sensitivity of sensory perception.

All of the *Leuconostoc, S. lactis,* and *S. cremoris* strains showed negligible CO_2 production when grown alone in milk. Some of the associative types showed gas production from citrate, a characteristic also possessed by three of the five *S. diacetilactis* cultures. The *S. lactis* and *S. cremoris* strains were all negative in this regard. Gas production from glucose was not accomplished by any of the *Leuconostoc.* Gibson and Abdel-Malek (5) have used this cultural test (gas from glucose) to identify *L. citrovorum, L. dextranicum,* and *L. mesenteroides.* The test did not appear selective in this study. In general, sugar fermentation studies provided inconclusive data with regard to taxonomy of the lactic streptococcus and *Leuconostoc* strains in the present investigation. The *L. mesenteroides* strain (P-60) was distinguishable from the dairy associative organisms on the basis of several cultural reactions; it hydrolyzed arginine and survived holding

TABLE 1

Cultural reactions of Leuconostoc and lactic streptococcus species representing stock strains and isolates from commercial mixed strain starters

Species	No. of strains tested	Milk coagulation in 48 hr. at 30° C.	Growth		Arginine hydrolysis	CO_2 produced in milk	Citrate utilization	Aroma production	
			40° C.	4.0% NaCl				Single strain	*Leuconostoc* plus lactic streptococcus
Stock strains									
Leuconostoc mesenteroides	1	−	+	+	+	L[c]	−	−	−
Leuconostoc species	12	−	−(2)	−(5)	−	L	−(4)	−	+(1)
Leuconostoc citrovorum	5	−(1)[b]	−(2)	+	−(1)	L	−	−	−(2)
Leuconostoc dextranicum	4	−	−(2)	+	−	L	−	−	−(2)
Streptococcus lactis	8	+	+	+(2)	+	L	−	−	
Streptococcus cremoris	13	+	−(4)	−(2)	−(1)	L	−	−	
Streptococcus diacetilactis	5	+	+	+	+(1)	H(1)	+(2)	+(1)	
Isolates[a]									
Streptococcus cremoris	18	+	−(3)	−	−	L	−	−	
Leuconostoc species	2	−	−	−	−	L	−	−	±
Streptococcus lactis var. aromaticus	2	+	−	−	+	L	−	±[d]	
Streptococcus diacetilactis	12	+	+	+	+(4)	H	+	+	

[a] When two or more strains isolated from a mixed-strain culture showed identical test reactions, one was selected for reporting purposes.

[b] Parentheses indicate number of strains of the total tested that show opposite reaction.

[c] L = low; H = high.

[d] ± Indicates variable results.

for 30 min. at 60° C., it grew at 10, 40, and 45° C., at pH 9.2 and 9.6, and in the presence of 4.0 and 6.5% sodium chloride.

All of the *S. diacetilactis* strains showed some properties of *S. lactis*. The A.T.C.C. 11007 strain, however, was atypical for the species, since no aroma compounds or CO_2 were produced in milk culture. Aroma compounds and CO_2 were produced by the four other strains.

Some of the *S. lactis* strains and two of the *S. diacetilactis* strains appeared to be capable of utilizing a number of carbohydrates without producing acid; visual and microscopic examination showed heavy growth, yet the brom cresol purple indicator remained unchanged. This also was accomplished by some of the isolates from starter cultures; here, however, it almost always was limited to the high CO_2-producing types. Three or more of the carbohydrates xylose, rhamnose, inulin, dextrin, and glycerol were involved in each case.

All the high CO_2-producing strains isolated from starter cultures gave identifying citrate fermentation reactions. The quantity of CO_2 produced by these organisms was so large that the agar seal plugs in tubes of inoculated Medium 1 (glucose + citrate) were forced up about 10 cm. The plugs in tubes of Medium 2 (citrate) were raised about 2 cm. Acid but no gas was produced in Medium 3 (glucose).

A large number of *S. cremoris* organisms were isolated from the mixed-strain starter cultures examined. No strains of *S. lactis* were found. Many high gas-producing organisms (*S. diacetilactis*) also were isolated from starter cultures, which re-emphasized the importance of these types in commercial cultures. *Leuconostoc* species were isolated from only one mixed-strain culture. This was accomplished by allowing the culture to incubate at 25° C. for 2 wk. before serial dilution plating. This same technique was used with the other mixed-strain cultures, but no *Leuconostoc* organisms were found. Two cultures isolated from this same mixed-strain culture were difficult to identify. They possessed typical *S. cremoris* cultural reactions, except that a slight positive King reaction was produced and flavor and aroma compounds were pronounced when the milk cultures were tasted. They were classified as low CO_2-producers, yet the tendency to produce slightly more gas than *S. cremoris* and *S. lactis* strains was evident. Subsequently, these two organisms were designated as *S. cremoris* var. *aromaticus*.

Serological results. The results of the cultural and biochemical tests suggested the advisability of determining to what Lancefield group the high gas-producing strains of lactic streptococci belonged. It was felt that these data would further insure the proper assignment of genus and species for these bacteria.

Positive precipitin reactions were obtained in about 30 sec. when the serum from rabbits immunized against *S. lactis* E was combined with cell extracts of *S. lactis* E or *S. lactis* 7962. Likewise, positive precipitin reactions were noted in the same time period when the serum from rabbits immunized against *S. lactis* 7962 was combined with cell extracts of *S. lactis* E or *S. lactis* 7962. These results indicated the presence of Group N antibodies in the rabbit sera in sufficient concentration to proceed with further tests to determine whether or not the high gas-producing organisms were members of Group N.

All of the known strains of *S. diacetilactis* gave positive precipitin reactions with both preparations of Group N sera, which substantiated the report of Briggs (2) that this organism is in Lancefield Serological Group N. Likewise, all of the high gas-producing organisms isolated from mixed-strain starter cultures gave positive precipitin reactions with the Group N sera.

Cell extracts from Leuconostoc species reacted negatively under the precipitin test with the Group N sera. *S. cremoris* C_3, suspected of being *S. fecalis* because of unusual reactions during the taxonomic study, however, gave positive tests.

DISCUSSION

A study of the complete taxonomic data for the known organisms revealed that certain of the tests were of limited value in differentiating the genera or species. Growth at pH 9.2 and pH 9.6, growth in 6.5% sodium chloride, as well as survival at 60° C. for 30 min. appear to be in this category. The sugar fermentation studies provided few data of taxonomic significance except, possibly, to show the greater fermentative abilities of the high CO_2-producing organisms. The strains designated as *L. citrovorum* or *L. dextranicum* which were available for use in this study could not be distinguished as either species by any of the tests performed. The hydrolysis of arginine by *L. mesenteroides* may be characteristic for this species and perhaps the test could be used to separate it from *Leuconostoc* isolated from raw milk supplies. A number of workers, for example Hammer and Baker (6) and Hucker and Pederson (7), have reported on some associative *Leuconostoc* types that were active gas-producers. In this study, however, all of the *Leuconostoc* species encountered showed negligible CO_2 production in milk culture.

The validity of most of Sherman's cultural tests (20) to distinguish between *S. lactis* and *S. cremoris* again was confirmed by this study. These two species easily were separated by the arginine hydrolysis test, along with observations for growth at 40° C. and in the presence of 4.0% sodium chloride. The arginine hydrolysis test appears the most reliable, as there were more inconsistent differences in the other reactions.

Classification of 18 of the organisms isolated from mixed-strain starter cultures as *S. cremoris* appears justified on the basis of comparison with the taxonomic data for the stock *S. cremoris* organisms (also shown in Table 1). All of these strains failed to hydrolyze arginine, all but three failed to grow at 40° C., and none grew in the presence of 4.0% sodium chloride. Furthermore, none of these organisms produced aroma compounds or significant amounts of CO_2 in milk.

S. cremoris var. *aromaticus* (9) is suggested as a possible designation for two of the isolates appearing in Table 1, while two others were classified as *Leuconostoc* species.

The remaining 12 strains were classified as *S. diacetilactis*. This name originally was used by Matuszewski *et al.* (12) to describe citric acid–fermenting streptococci other than *Leuconostoc*. According to their description, *S. diacetilactis* grows at 10 and 40, but not at 45° C.; it survives 30 min. at 60° C.; in milk it produced dextrorotatory lactic acid along with CO_2 and flavor compounds in

large quantity; glycerol, mannitol, xylose, sucrose, raffinose, and inulin are not fermented; acid is produced from arabinose, dextrose, lactose, maltose, dextrin, and salicin.

The 11007 strain of *S. diacetilactis* appeared different from the original Matuszewski strain, since it was unable to produce C-4 compounds and CO_2 in milk. The loss of these two characteristics may be due to prolonged culturing on media other than milk.

Classification as *S. diacetilactis* of the 12 newly isolated strains included in this study appears reasonable when their cultural reactions are compared with those of the known species (also shown in Table 1) and with the original description of this organism (12). All of them produce C-4 aroma compounds and large quantities of CO_2 in milk and are capable of fermenting citrate. Furthermore, support for the conclusion that the high gas-producing organisms isolated from mixed-strain starter cultures are *S. diacetilactis* was obtained from results of the serological studies.

Data obtained in this study may aid in identification of the four types of single-strain lactic acid streptococci isolated from mixed-strain starter cultures as described above and in an earlier communication (18). The high acid–high gas and low acid–high gas types appear to be *S. diacetilactis* organisms. The latter type frequently is found in commercial starter cultures, while the former type is isolated less often. Only one strain of the high acid–high gas type was encountered in this study. The high acid–low gas strains appear to be *S. lactis* or *S. cremoris*, which species are easily separated by testing for hydrolysis of arginine. The low acid–low gas types are more difficult to classify, because they may be either slow acid-producing strains of *S. lactis* or *S. cremoris*, or they may be *Leuconostoc* species. Separation of lactic streptococcus and *Leuconostoc* species was accomplished in this study by culturing the organisms at 30° C. in litmus milk; members of the *Streptococcus* genus caused acid coagulation in 48 hr., whereas *Leuconostoc* produced little or no acid under these conditions. It should be remembered also that *S. lactis* var. *aromaticus* or *S. cremoris* var. *aromaticus* may be found, possibly as intermediate forms between *S. lactis* or *S. cremoris* and *S. diacetilactis*.

The presence of *S. diacetilactis* organisms in mixed-strain starter cultures may have been due in some instances to the belief that they were *Leuconostoc* types. The slow rate of acid production by most strains of this species, particularly at 21° C., relates them to the associative types.

The possibility that the presence of *S. diacetilactis* strains in cultures used for the manufacture of Cheddar cheese may lead to the split-open defect has been considered (18). Robertson (15) also has indicated that this organism is involved in openness in Cheddar cheese, because of its CO_2-producing ability. It also is possible that other instances of slit openness in Cheddar cheese may have been caused by this organism (14).

Studies on the metabolism of *S. diacetilactis*, using radioactive tracer techniques, have been initiated. Preliminary results indicate that cells of this organism growing in milk may produce CO_2 from the one, three, and four carbons of glucose and also from one or more of the carboxyl carbons of citric acid.

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STUDIES ON THE FLAVOR OF CREAMED COTTAGE CHEESE [1]

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SUMMARY

The biacetyl contents of 41 samples of commercial creamed cottage cheese, obtained from retail stores, varied from 0 to 3.2 p.p.m.; 73.1% of samples contained less than 1.0 p.p.m. and 95.1% contained less than 2.0 p.p.m.

When cottage cheese is manufactured by the long-set method, the maximum amount of biacetyl capable of being produced by a lactic culture is not attained at the time of cutting the curd (pH 4.7). During cottage cheese manufacture there is a partition of the milk constituents and flavor compounds. The whey fraction contains considerably more citric acid, lactose, biacetyl, and acetylmethylcarbinol than the curd. However, a proportionate amount of the biacetyl present at the time of cutting is retained by the cheese and the use of a culture producing considerable biacetyl results in cheese with a correspondingly higher biacetyl content.

The addition of citric acid to a creaming mixture for cottage cheese did not result in an increased biacetyl content when the cheese was held at 45° F. The addition of both citric acid and lactic culture to a creaming mixture for cottage cheese increased the biacetyl content when the holding temperature was 45° F., but a sour flavor was evident after several days' storage.

The dairy industry is striving constantly to manufacture uniform dairy products. The factors which must be considered in judging uniformity vary somewhat among the different dairy products. With most products, flavor, composition, and physical characteristics are important considerations. Much progress has been attained in the manufacture of creamed cottage cheese with uniform composition and physical characteristics. Less progress has been made in standardizing the flavor. This is indicated rather clearly by the data in Table 1, which show the biacetyl contents of 41 samples of creamed cottage cheese obtained from retail stores. Similar data have been reported by other investigators (5, 9).

TABLE 1

Biacetyl contents of 41 samples of creamed cottage cheese

Biacetyl content	No. of samples	Biacetyl content	No. of samples
(p.p.m.)		*(p.p.m.)*	
0.0	2	0.9	1
0.1	2	1.1	1
0.2	9	1.2	2
0.3	3	1.3	3
0.4	4	1.4	1
0.5	5	1.9	2
0.6	1	2.6	1
0.7	2	3.2	1
0.8	1		

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It is recognized that biacetyl is not the only flavor contributor to creamed cottage cheese but that it is an important one (5, 6, 9). The studies reported here were designed to provide information on various factors which influence the biacetyl content of creamed cottage cheese.

METHODS

Biacetyl and acetylmethylcarbinol. The colorimetric method of Prill and Hammer (8) was used for the estimation of biacetyl and acetylmethylcarbinol in cultures and cottage cheese. Color intensity was measured with a Beckman spectrophotometer (Model B) at a wave length of 530 mμ. Dimethylglyoximate was used in constructing the standard curve.

Citric acid. The chromatographic method of Bulen *et al* (1), as modified by Frazeur (2), was used for the estimation of citric acid.

Lactose. The method of Perry and Doan (7) was used to determine lactose in milk, culture, whey, and cottage cheese. Color intensity was measured with a Beckman spectrophotometer (Model B) at a wave length of 520 mμ.

Volatile acids. A 50-g. sample of product was steam-distilled after the addition of 3 ml. of 1 *N* sulfuric acid. The first 100 ml. of distillate was titrated with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator.

Manufacture of cottage cheese. Cottage cheese was made according to the long-set method outlined by Hales (3). When creamed, a 12% fat creaming mixture was added at the rate of one part creaming mixture to two parts cheese. The creamed cheese was salted at the rate of 1%.

RESULTS

Fermentation products formed by lactic cultures in skimmilk at 70° F. Lactic cultures grown in skimmilk produce several compounds which have an effect on flavor. The rates of formation of lactic acid, biacetyl, and volatile acids, all of which influence flavor, are shown in Table 2. The amounts of lactose and citric acid fermented, as well as the amount of acetylmethylcarbinol formed, are given also. Rate of acid production is shown by titratable acidity and pH determinations. A decrease in lactose occurred as the titratable acidity increased and pH decreased, but even after 24 hr. of incubation considerable lactose was still present. Biacetyl increased very little during incubation for 10 hr., but for the next 6 hr. appreciable amounts were formed. Acetylmethylcarbinol increased throughout the incubation period; however, large increases were evident only after 8 hr. of incubation. Volatile acids also accumulated throughout the incubation period and large increases were noted after 10 hr. of incubation. The citric acid content decreased throughout the incubation period and only 0.02% remained after 24 hr.

Partition of citric acid, lactose, biacetyl, and acetylmethylcarbinol during the manufacture of cottage cheese. The data presented in Table 3 indicate how certain milk constituents and fermentation products are partitioned between the curd and whey. Two trials are included, one with a culture which produced considerable biacetyl, and the other with a culture which produced only a small

TABLE 2
Fermentation products formed by a lactic culture in skimmilk at 70° F.

Incubation time	Titratable acid	pH	Lactose	Biacetyl	Acetylmethylcarbinol	Volatile acid	Citric acid
(hr.)	*(%)*		*(%)*	*(p.p.m.)*	*(p.p.m.)*	*(ml. 0.1 N NaOH)*	*(%)*
0	0.18	6.44	5.3	0.2	1.0	0.18	.....
1	0.18	6.42	5.3	0.2	1.2	0.20	0.14
2	0.19	6.38	5.3	0.1	1.5	0.25	.....
3	0.19	6.29	5.3	0.1	1.7	0.27	0.13
5	0.22	6.27	5.3	0.2	1.8	0.37	0.12
6	0.26	6.10	5.1	0.1	1.9	0.39	.....
7	0.30	5.81	5.2	0.3	2.7	0.52	.....
8	0.35	5.55	5.1	0.4	3.0	0.55	0.11
9	0.44	5.37	5.1	0.3	7.1	0.63	.....
10	0.52	5.15	5.0	0.5	13.1	0.70	0.09
12	0.68	4.83	4.9	1.1	30.1	1.20	0.09
13	0.77	4.69	4.5	1.5	42.9	1.47	.....
14	0.82	4.59	4.5	2.1	57.9	1.72	0.08
15	0.84	4.58	4.5	2.3	89.7	2.12	.....
16	0.89	4.56	4.5	2.8	97.2	2.73	0.05
24	0.97	4.44	4.4	2.5	103.5	3.52	0.02

amount. In both lots, much of the citric acid was fermented prior to cutting the coagulum. The remaining citric acid was removed largely with the whey. In Lot 1, 15.1% of the lactose was fermented prior to cutting, and 9.5% in Lot 2. Although most of the lactose was contained in the whey fraction, a considerable portion was present in the washed and drained curd. The biacetyl content of the coagulum just prior to cutting, and of the whey, were practically identical, indicating that biacetyl formed by the fermentation of citric acid was removed largely by the whey fraction. The curd contained less than one-half the percentage of biacetyl contained in the whey. Partition of acetylmethylcarbinol followed the same general trend as biacetyl.

Effect of addition of citric acid to creamed cottage cheese on the biacetyl content. Since biacetyl is formed by fermentation of citric acid in milk by lactic cultures, an experiment was conducted to determine the effect of addition of citric acid to creamed cottage cheese on the biacetyl content. A batch of cottage cheese was divided into three lots and treated as follows: Lot 1, no treatment;

TABLE 3
Partition of citric acid, lactose, biacetyl, and acetylmethylcarbinol during the manufacture of cottage cheese

Product	Citric acid *(%)*		Lactose *(%)*		Biacetyl *(p.p.m.)*		Acetylmethylcarbinol *(p.p.m.)*	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
Skimmilk	0.13	0.14	5.3	5.3	....	....	....	....
Culture	....	....	....	....	2.8	0.6	94.8	82.4
Coagulum prior to cutting	0.05	0.08	4.5	4.8	2.3	1.1	55.7	30.1
Whey	0.08	0.09	4.7	4.9	2.3	1.2	52.7	22.8
Washed curd	0.01	0.04	1.8	2.0	1.0	0.5	29.0	19.0
Thoroughly drained curd	0.01	0.04	1.7	2.0	1.0	0.6	24.2	16.2

Lot 2, one part by weight of creaming mixture containing 12% fat was added to two parts cottage cheese; Lot 3, treated the same as Lot 2 except that 0.15% citric acid was added. The citric acid was added as a 15% solution. Samples of each lot were stored at 45° F. and analyzed at intervals for biacetyl, acetylmethylcarbinol, and pH. Results of these analyses are given in Table 4.

TABLE 4

Effect of addition of citric acid to creamed cottage cheese on the biacetyl content

Days held at 45° F.	Lot 1 Cottage cheese			Lot 2 Creamed cottage cheese			Lot 3 Creamed cottage cheese plus citric acid		
	Biacetyl	Acetylmethylcarbinol	pH	Biacetyl	Acetylmethylcarbinol	pH	Biacetyl	Acetylmethylcarbinol	pH
	(p.p.m.)	*(p.p.m.)*		*(p.p.m.)*	*(p.p.m.)*		*(p.p.m.)*	*(p.p.m.)*	
0	1.4	16.4	4.57	1.2	6.2	4.88	1.0	4.6	4.65
2	1.5	16.5	4.69	1.2	12.0	4.91	1.1	12.1	4.71
4	1.4	15.6	4.64	0.9	9.1	4.91	1.0	13.4	4.69
6	1.5	18.1	4.65	1.2	11.0	4.93	1.0	13.8	4.70
8[a]	1.4	17.8	4.65	1.2	10.0	4.85	1.2	21.2	4.79
13[b]	1.2	5.6	4.79	0.9	5.7	4.88	0.8	12.8	4.69

[a] Lot 2, slightly sour; Lot 3, sour.
[b] All lots spoiled.

Lot 1 remained quite constant in biacetyl content throughout the 13-day holding period. Only small fluctuations were noted in the acetylmethylcarbinol content for eight days, but a large decrease occurred at 13 days. The pH increased from 4.57 to 4.79 during the holding period. Lot 2 also remained quite constant in biacetyl, but the acetylmethylcarbinol content fluctuated. An increase in carbinol was evident after two days and a large decrease at 13 days. The pH at 0 and 13 days' storage was identical. Lot 3, with added citric acid, remained constant in biacetyl content for eight days and then decreased. The carbinol content increased for eight days and then a large decrease was apparent. The pH showed only slight variations.

Effect of addition of citric acid and lactic culture to creamed cottage cheese on the biacetyl content. The failure to obtain a large increase in biacetyl when citric acid was added to creamed cottage cheese suggested the lack of a sufficient number of organisms capable of fermenting the acid. Therefore, an experiment was conducted to study the effect of additions of citric acid and lactic culture. The culture selected produced considerable biacetyl in cultured buttermilk. The data obtained in this experiment are given in Table 5. Lots 1, 2, and 3 were prepared in the same manner as in the previous experiment. Lot 4 was similar to Lot 3, except that the creaming mixture contained 2% lactic culture. All lots were held at 45° F. and analyzed for biacetyl, acetylmethylcarbinol, and pH at various intervals.

The cottage cheese used in the preparation of these lots contained an appreciable amount of biacetyl and when held uncreamed (Lot 1) it maintained approximately the same biacetyl content. The acetylmethylcarbinol content increased slightly for six days and then decreased to its approximate original

TABLE 5

Biacetyl content of cottage cheese, creamed cottage cheese, creamed cottage cheese plus citric acid, and creamed cottage cheese plus citric acid and lactic culture

Treatment	Days held at 45° F.														
	0	2	4	6	9	0	2	4	6	9	0	2	4	6	9
	Biacetyl					Acetylmethylcarbinol					pH				
	(p.p.m.)					*(p.p.m.)*									
Lot 1. Cottage cheese	1.3	1.5	1.5	1.4	1.4	6.9	7.6	10.1	10.4	6.4	4.77	4.80	4.76	4.75	4.89
Lot 2. Creamed cottage cheese	1.0	1.0	1.1	1.0	1.0	6.6	8.3	5.4	7.6	11.6	5.10	5.08	5.14	5.16	5.14
Lot 3. Creamed cottage cheese plus citric acid	1.0	1.0	1.0	0.8	0.8	6.6	7.0	7.3	6.2	5.7	4.76	4.80	4.82	4.83	4.83
Lot 4. Creamed cottage cheese plus citric acid and lactic culture [a]	0.8	1.8	3.1	2.9	2.2	9.0	27.6	82.4	78.1	34.8	4.80	4.68	4.43	4.50	4.46

[a] Lot 4 sour at four days and thereafter.

value at nine days. The pH remained quite constant for six days, with a definite increase being noted at nine days. The addition of cream to cottage cheese (Lot 2) decreased the biacetyl content by about one-third, the extent of dilution by the creaming mixture. Slight variations were found in the carbinol content, the most important being an increase at nine days. No apparent change occurred in pH. The addition of citric acid to the creaming mixture (Lot 3) did not give an increase in biacetyl or carbinol, but it did decrease the pH by about 0.3 unit. Addition of both citric acid and lactic culture to the creaming mixture (Lot 4) resulted in an increase in biacetyl which reached a maximum in four days, then decreased. The carbinol content showed exactly the same trend as the biacetyl content. The pH decreased after holding for four days and a sour flavor was noted at this time.

DISCUSSION

The flavor imparted by biacetyl to dairy products such as butter, cultured buttermilk, cultured sour cream, and various cheeses is considered desirable. However, the biacetyl content of dairy products has not been well standardized and this is particularly true of cottage cheese. The data in Table 1 confirm this statement and show a range of 0 to 3.2 p.p.m. biacetyl in retail samples of cottage cheese. A biacetyl content of about 2 p.p.m. gives a pleasing flavor and seems to mask a sour flavor sometimes associated with cottage cheese.

Although considerable research has been conducted on the chemical compounds produced by lactic cultures growing in milk (4), the literature does not contain a complete analysis showing the amounts of lactose and citric acid fermented and changes in pH, titratable acidity, biacetyl, acetylmethylcarbinol, and volatile acids as a result of these fermentations. Such an analysis is presented in Table 2.

The first stage of cottage cheese manufacture by the long-set method is quite similar to the manufacture of cultured buttermilk, since with both products heated skimmilk is inoculated with lactic culture and incubated at 70° F. The data presented in Table 2 were intended to show that with cultured buttermilk ripened to 0.80 to 0.85% titratable acidity, considerably more of the flavor compounds (biacetyl, volatile acids, etc.) are produced than at the cutting time for cottage cheese (pH 4.7). Also, with cottage cheese there is a partition of the fermentation products and other milk constituents between the curd and whey. Such a partition does not occur in the manufacture of cultured buttermilk.

The biacetyl content of the coagulum prior to cutting has an influence on the biacetyl content of cottage cheese. A proportionate amount of the biacetyl in the coagulum is retained by the cheese. This is shown by the data in Table 3, which were obtained by cultures having different capacities to produce biacetyl. The fermentation of citric acid during setting of the cheese, and the loss of most of the remaining citric acid in the whey, result in cottage cheese having only a small amount of the compound from which to form additional flavor materials. Numerous studies (4) have indicated that citric acid is the source of biacetyl.

The biacetyl content of cottage cheese held at 45° F. remains quite constant for several days. When creamed, the biacetyl content also remains quite con-

stant, but it is decreased in proportion to the dilution with cream. The citric acid or citrates contained in the creaming mixture, as well as citric acid added to such a mixture, were not fermented to yield additional biacetyl. Failure to obtain an increase in biacetyl may be due to too high a pH, small numbers of citric acid–fermenting organisms, failure of the organisms contained in the curd particles to reach the substrate, an unfavorable temperature, and/or perhaps other factors.

The addition of lactic culture to a creaming mixture containing added citric acid (Table 5) increased the biacetyl content of creamed cottage cheese. The increase from an initial content of 0.8 p.p.m. to 3.1 p.p.m. in four days had a definite effect on flavor. However, the lactic culture also produced sufficient lactic acid during the holding period to give the cheese a sour flavor. Perhaps a lower holding temperature would have decreased the amount of lactic acid produced, but 45° F. is a common holding temperature for cottage cheese in retail stores and in the home.

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THE GROWTH OF STAPHYLOCOCCI IN CONDENSED SKIMMILK [1]

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SUMMARY

The following variables were studied from the standpoint of their effect on growth of staphylococci in condensed milk: *(a)* precondensing heat treatments, *(b)* milk solids concentrations, and *(c)* growth temperatures. Growth also was studied under conditions simulating vacuum-condensing operations.

Preheat treatment of skimmilk at 165 or 185° F. for 30 min. before condensing did not affect the growth of two cultures studied. However, growth of one of these cultures was retarded somewhat when the preheat treatment was 150° F. for 30 min.; growth of the other culture was not retarded.

Cultures of staphylococci grew optimally, or nearly so, at temperatures ranging from 90 to 113° F. in condensed skimmilks having solids concentrations ranging from approximately 30 to 50%. Three of six cultures grew, although slowly, in 40% milk solids at 116° F. and one grew under similar conditions at 118° F.

Under conditions of simulated vacuum condensing, growth was less extensive than under normal atmospheric pressure. However, growth was sufficiently rapid to exclude the possibility that subatmospheric pressures, likely to prevail in a vacuum-condensing operation, would afford sufficient retardation of growth to be of any practical significance in controlling growth of staphylococci.

During recent years, outbreaks of staphylococcal food poisoning have implicated nonfat dry milk. In England, a series of eight outbreaks, all associated with foods prepared with dried milk, were reported by Anderson and Stone (2). In 1956, a series of 19 outbreaks of gastroenteritis among school children participating in the school lunch program in Puerto Rico was reported by Armijo *et al.* (3). Although bacteriological and toxicological tests for metallic poisons were negative, clinical and epidemiological evidence indicated that staphylococcal enterotoxin was the probable agent.

Various opinions have been expressed as to the probable sources of staphylococci and conditions for their growth in milk and condensed milk for drying. Anderson and Stone (2) and Hobbs (7) have reported that raw milk, heated milk, or condensed milk may show considerable staphylococcal growth and probable enterotoxin production. Hobbs (7), Hawley and Benjamin (5), Crossley and Campling (4), and Heineman (6) were of the opinion that extensive growth and consequent enterotoxin formation most likely would occur during the manufacturing process following preheating of the milk.

Heineman (6) has reported that pasteurized milk and condensed skimmilk (29.02–42.77% solids) served as excellent media for the growth of *Staphylococcus aureus*. He pointed out that whether enterotoxin formation occurred could only be speculative, since no tests for enterotoxin were made. Crossley and Campling (4) reported that *S. aureus* always proliferated in condensed whole milk (38–40% solids) following inoculation of the product after the condensing

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process. When the milk was inoculated before condensing, they observed that the population of *S. aureus*, in most trials, was reduced during the vacuum-condensing process. However, the condensing process was relatively short, being 4 to 5 hr. Upon further incubation of this condensed milk at 37° C., the staphylococcal count was further reduced. They offered no explanation for the failure of growth in the inoculated milk during and after condensing.

The present study was undertaken to determine *(a)* the effect of precondensing heat treatment of skimmilk on growth of *S. aureus* in condensed skimmilk; *(b)* the effect of various incubation temperatures and skimmilk solids concentrations on the growth of *S. aureus* in condensed skimmilk, and *(c)* the effect of conditions simulating those which may occur during vacuum condensing on the growth of *S. aureus*.

MATERIALS AND GENERAL METHODS

One of the cultures of *S. aureus* designated as 196E in the figures presented herein was enterotoxigenic and was obtained from the Robert A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati, Ohio. Other cultures of this species used in these studies were obtained from various sources; all were coagulase-positive but may or may not have been enterotoxigenic.

Milk used was heated to 110° F. and separated. Following separation, the skimmilk was immediately heated to 185–188° F., held for 30 min., and cooled to 40° F. In preliminary studies, the skimmilk was heated to 150, 165, and 185° F. for 30 min. to determine the effect of precondensing heat treatment on growth of staphylococci in the condensed product. In all experiments, the skimmilk was condensed to the desired milk solids concentration in a "Flash-Evaporator."[2] To prevent foaming and loss of product during condensing, 0.01 ml. of sterile Antifoam B (Dow Corning) was added per liter of skimmilk. The vapor temperature in the evaporating flask during condensing ranged between 36 and 54° F.

In the various experiments, growth under conditions described was measured by the agar plate method (1), using Plate Count Agar (Difco), Staphylococcus Medium No. 110 (Difco), and an incubation temperature of 37° C. for 48 hr. The two media revealed essentially the same information; consequently, only the results obtained through use of the Staphylococcus Medium No. 110 are presented.

EXPERIMENTAL METHODS AND RESULTS

Effect of precondensing heat treatment of skimmilk on growth of staphylococci in condensed skimmilk. Condensed skimmilks of 40% solids prepared from skimmilks given three different precondensing heat treatments were placed in 8-oz. bottles containing 40–60 glass beads. The condensed milks were inoculated with the respective culture, using sufficient inoculum to obtain a population of approximately 10,000 per milliliter in the inoculated product. The bottles were then incubated at 99° F. At 3-hr. intervals, the bottles were removed from the incubator, shaken vigorously and plated.

[2] Laboratory Glass and Instrument Corporation, New York, N. Y.

Figure 1 illustrates the growth of Cultures 196E and 1363 at 99° F. in condensed milks prepared from skimmilks subjected to precondensing heat treatments of 150, 165, and 185° F. for 30 min. The data indicate that rapidity of growth of Culture 196E in the three products was essentially the same. Culture 1363 responded similarly, except for a noticeable retardation of growth, which occurred in the condensed product prepared from skimmilk preheated to 150° F. for 30 min. The higher precondensing heat treatments more closely approximate those which are used commercially. Consequently, the skimmilks used in subsequent experiments were all heated to 185° F. for 30 min. before condensing.

Growth of staphylococci in condensed skimmilks of various milk solids concentrations and at various incubation temperatures. Condensed skimmilks containing 30, 41.5, and 47% milk solids were placed in 8-oz. bottles containing glass beads. The condensed products were inoculated as indicated in the previous experiment. The bottles were incubated at 90, 99, and 108° F. Platings were made at 3-hr. intervals.

In Figure 2, the growth curves for Culture 196E at the three temperatures indicated above and in condensed skimmilk containing 47% milk solids are shown. Essentially the same data were obtained for this culture when grown in condensed skimmilk containing 30 and 41.5% milk solids and, therefore, are not presented. The data represented by these curves show that growth was rapid at the three temperatures used and they indicate that the optimum growth temperature was at least 108° F. The data further show that the concentration of milk solids up to 47% had little effect on the growth of this culture.

A similar study was made using another strain of *S. aureus*. Data obtained are not presented, because they were essentially the same as those obtained for Culture 196E, except that growth at 90° F. was somewhat slower than that for Culture 196E at this temperature. As in Culture 196E, the concentration of milk solids had little effect on growth.

In additional experiments, condensed milk was standardized at 40% milk solids.

Figure 3 shows the results obtained with Culture 196E when incubated in water baths at 113, 116, 118, and 120 $\pm$ 0.5° F. Growth was rapid at 113° F. At 116° F., death of many cells occurred after 3 hr., but an initiation of limited growth occurred after 9 hr. and continued until 18 hr. Growth was completely inhibited at 118 and 120° F.

Following the studies with Culture 196E, growth of five additional strains of *S. aureus* in condensed milk was studied at 90, 99, 113, 116, and 118° F. Two of these five cultures also were grown at 108° F. A portion of the data obtained using these cultures as well as Culture 196E (indicated as No. 2) is presented in Figure 4. A comparison of the populations reached in 12 hr. at each of the six temperatures is shown. Three cultures failed to grow at 116° F., while three, including Culture 196E, showed limited growth. One of the five cultures grew at 118° F.; all others failed to grow.

Growth of staphylococci in condensed milk during the vacuum-condensing process. In these experiments, the growth of Culture 196E in condensed milk

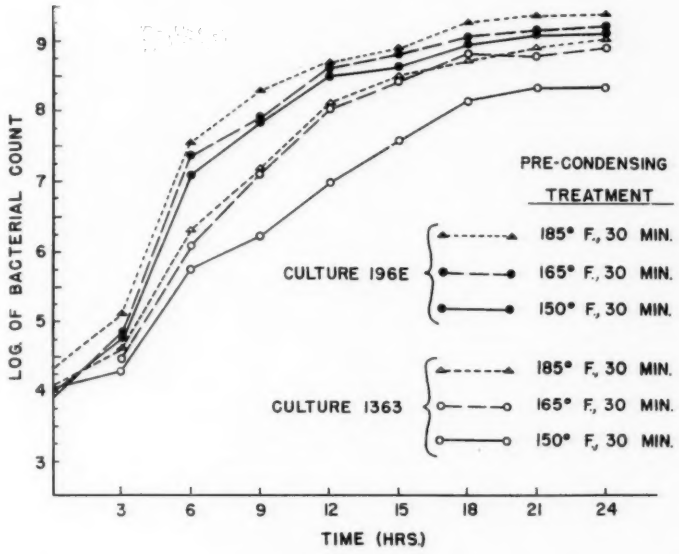

FIG. 1. Effect of precondensing heat treatments on the growth of Cultures 196E and 1363 at 99° F. in condensed skimmilk of 40% solids.

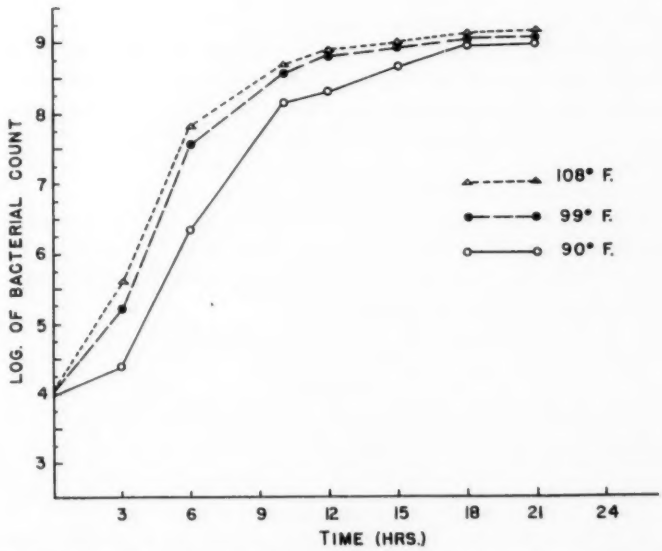

FIG. 2. Growth curves for Culture 196E at various temperatures in condensed skimmilk of 47% solids.

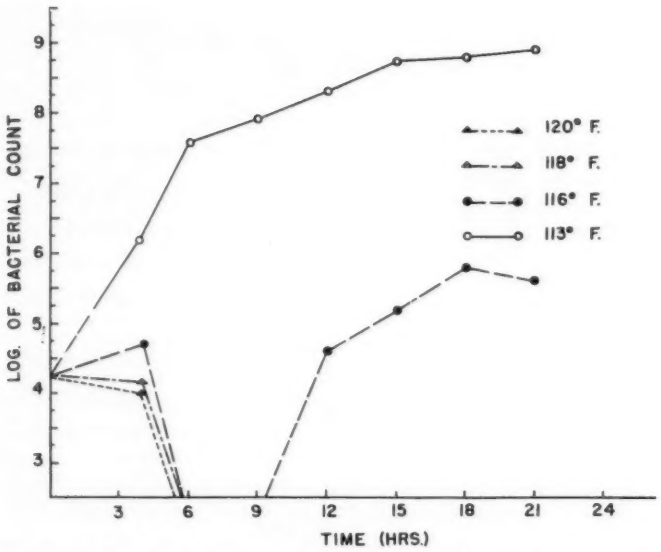

FIG. 3. Growth curves for Culture 196E in condensed skimmilk (40% solids) at various temperatures.

(40% milk solids) during the condensing procedure was studied. In the various experiments, the desired temperature of the condensed skimmilk during continuous operation of the evaporator was maintained by regulating the absolute pressure. A preliminary study established the relationships shown in Table 1.

The heating bath was maintained at a temperature only slightly higher than the desired boiling temperature of the condensed skimmilk in the evaporating flask. Therefore, the rate of evaporation was kept to a minimum. Sterile distilled water was continuously added to the condensed skimmilk to further maintain a uniform concentration throughout the growth period.

After inoculation with Culture 196E, 500 ml. of the condensed skimmilk (40% solids) were placed in the evaporator flask and the apparatus was put into operation. An additional 500 ml. were placed under normal incubation. At 3-hr. intervals, samples were removed and plated.

Figure 5 illustrates the growth at 108° F. which occurred under the condi-

TABLE 1
Temperature and pressure relationships during condensing processes

Boiling temperature of condensed skimmilk[a]	Temperature of heating bath	Absolute pressure[b]
(° F.)		(*Cm. Hg.*)
99	100–102	5.0
108	109–111	6.5
113	114–116	7.5–7.8

[a] Thermocouple measurements.
[b] Manometer readings.

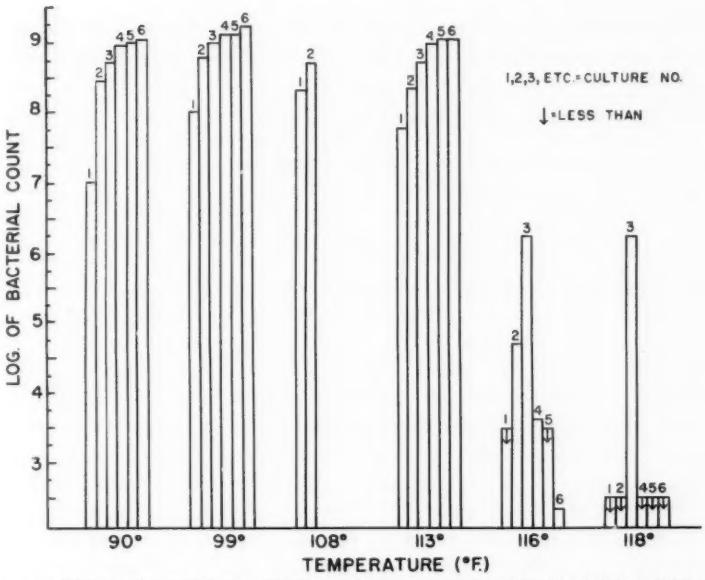

FIG. 4. Extent of growth of staphylococci in condensed skimmilk (40% solids) after 12 hr. of incubation at various temperatures.

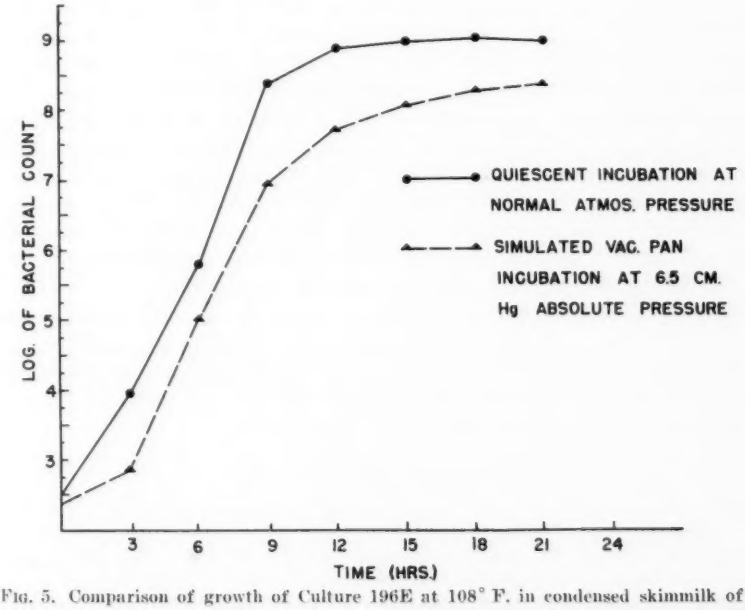
LOG. OF BACTERIAL COUNT
TIME (HRS.)
QUIESCENT INCUBATION AT NORMAL ATMOS. PRESSURE
SIMULATED VAC. PAN INCUBATION AT 6.5 CM. Hg ABSOLUTE PRESSURE

FIG. 5. Comparison of growth of Culture 196E at 108° F. in condensed skimmilk of 40% solids under conditions simulating vacuum-pan operation and under conditions of quiescent incubation at normal atmospheric pressure.

tions simulating vacuum-pan operation and under conditions of quiescent incubaton at normal atmospheric pressure. Rapid growth occurred under both conditions; however, under reduced pressure, growth lagged in the beginning and the final population was lower than that reached at normal atmospheric pressure. Reasons for these observed differences are yet to be determined. Growth curves also were obtained at 99 and 113° F. under the same conditions. Results were essentially the same.

DISCUSSION

Certain samples of condensed skimmilk in which *S. aureus* had grown to various population levels have shown some evidence of toxicity by a modified kitten test (8); however, proof of the presence of enterotoxin by this method is not unequivocal. The results obtained in the studies herein reported should not be interpreted as implying that enterotoxin was formed in the various samples. On the other hand, it should be recognized that growth of enterotoxin producing staphylococci does admit of the possibility of enterotoxin production.

During the manufacture of dried milks, the fluid products involved may vary in milk solids concentration from that of normal whole milk and skimmilk to approximately 50%. The temperatures to which these fluid products may be subjected may range from approximately 40 to 200° F.; temperatures intermediate within this range commonly occur. For example, it is common for condensed products in the last effect chamber of the vacuum condensing apparatus to be at temperatures ranging from 90 to 113° F. These temperatures are optimum, or nearly so, for the growth of staphylococci. Furthermore, milk solids concentrations or subatmospheric pressures likely to prevail during processing operations do not seem to retard appreciably the growth of staphylococci. Therefore, if enterotoxigenic staphylococci would be present, there may be an opportunity for growth not only within the vacuum pan system itself but also in lines and surge tanks, where condensed milk may be held prior to the final heat treatment or drying. This could result in the development of high populations of staphylococci and potentially, at least, in the formation of enterotoxin. Because of its heat stability and resistance to drying, enterotoxin once formed would, in all probability, be carried over into the dried milk.

Every precaution, therefore, should be taken to eliminate plant practices which might permit: *(a)* underpasteurized milk reaching or leaving the condensing apparatus, *(b)* the holding of condensed milk for any extended period at temperatures within the growth range of staphylococci, and *(c)* direct contamination of condensed milk with viable staphylococci.

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ESTABLISHING AGE-CORRECTION FACTORS RELATED TO THE LEVEL OF HERD PRODUCTION[1]

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SUMMARY

Age-correction factors have been developed based on the level of herd production. They are additive factors, expressed as regressions on the age-corrected herd average and, as such, might be termed within-herd additive gross comparison factors. They indicate that age corrections should be larger in herds of high production than in herds of low production, independent of the actual production of the young cow. One comparison with the multiplicative factors indicates that the latter do take account of between-herd differences in age effects. Work is in progress on comparing different sets of age-correction factors.

Comparisons between cows frequently entail animals of different ages and, since cows generally produce less when young than when fully grown, it is necessary to make allowance for age differences in these comparisons. Methods of adjusting records for this purpose are usually in the form of age-correction factors applied to the records of young cows. For this purpose, age is being taken as a measure of maturity and the factors are used to correct for stage of maturity. Lush and Shrode (15) state that "It seems impossible to determine differences in rate of maturity for individual cows, although it can be done for genetically distinct groups." However, it does not seem necessary to confine oneself to groups which are genetically distinct for, if rate of maturity is at all affected by environment, a study of groups which differ in environment, such as herds, is indicated. Most studies of age-correction factors have treated the effects of age as being peculiar to the cow itself, independent of its herd environment and, where consideration has been given to herd differences, it has been only to eliminate them from the data prior to estimating the age-correction factors, not to use them in actually estimating the factors. Several methods have been used in this manner.

Gowen (6 and 7) first developed a method of establishing age-correction factors based on comparing averages of all records available in different age groups, known as the Gross Comparison method. Its weakness, that of not necessarily comparing lactations of the same cows, was first circumvented by Sanders (17), using what has come to be known as the Paired Comparison method, in which comparisons are made for each consecutive pair of records, between lactations of the same group of cows. The biases in these methods are discussed by Lush and Shrode (15) and by Henderson *et al.* (10). A maximum likelihood method for estimating changes in herd environment is given by Henderson (8). This has been used by Beardsley (1) for estimating age effects in an investi-

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[1] Results presented in this paper are taken from a thesis submitted by the first author, in partial fulfillment of the requirements of the Ph.D. degree.

gation made by the American Jersey Cattle Club, the results of which have been published in the Jersey Bulletin (12).

Kay and M'Candlish (14) suggest that, in trying to find the effect of age on production, age is being used as a measurement of maturity, because the point of interest is the effect on production of the growth and bodily development of a cow from youth to old age. A cow's body size increases as it ages, likewise its mammary glands and udder, leading in general to greater milk production. These effects are combined with that due to the development of the milk secretory tissues with increasing age, particularly during the first and second lactations for, when a cow starts its second lactation, the functional activity of its alveoli has already been developed and stimulated by the processes of the first. This is an effect which occurs concurrently with age so that, to the extent that age is confounded with lactation number (i.e., first lactation at 2 yr. of age, second at 3 yr., and so on), age effects include effects due both to growth and development of the mammary system and to the cow's over-all stage of maturity. This is governed to some extent by herd environment and herd management, particularly by calf-raising methods and age-at-first-lactation practices, and it suggests taking herd effects into account when establishing age-correction factors.

In Beardsley's report (1), herd effects have been considered simultaneously with age effects; but once estimated, they are not used for the setting up of age-correction factors, and in no case has there been an investigation of to what extent, if any, age-correction factors should differ from herd to herd. A few workers have briefly mentioned the problem. Ward and Campbell (19) emphasize the necessity of having factors applicable to ordinary herd conditions, and suggest that the bias in using regression factors is more uniform than in using multiplicative ones. Dickerson (3) shows high among-herd correlations between dry period and calving interval, and between lactation length and calving interval. This suggests that to the extent to which these things affect age corrections they might be accounted for by studying herd differences. Dickerson and Chapman (4) took some account of differences in herd levels by stratification, whereas Gaines *et al.* (5) showed that the ratio of fat-corrected milk to weight was independent of age, both between and within herds. Mahadevan (16) overcame the difficulty of significant differences between herd levels by expressing each record as a deviation from the herd average. Hickman and Henderson (11) show that increases from first to second lactation vary greatly over herds, whereas in Castle's (2) data the within-herd difference between young and mature cows is greater in high-level herds than in low-level ones, suggesting that age corrections might be expressed as regressions on herd level. A method for doing this is now presented.

Method of analysis. Age-correction factors are designed to correct the records of young cows to what they would have been had the cows been older or more mature, all other environmental influences remaining the same. In this study, mature cows have been defined as those aged 5–9.5 yr., production over these ages being comparatively stable. Age-correction factors in this study

have, therefore, been designed to correct the records of young cows up to this stage of maturity. Corrections for cows older than 9.5 yr., as well as for younger ones could also be made, but they are so few in number in most herds that little is lost by omitting them.

When establishing age-correction factors as regressions on herd level, the question immediately arises of how to get a herd average free of age effects. The average of the actual production records for a herd composed entirely of young cows, 2-yr.-olds, say, is a downwardly biased estimate of the true mature equivalent herd level; on the other hand, the average production of a herd of entirely mature cows is free of such biases. As required here, the measure of the herd level must be free of these biases in all cases, implying the need of a herd average in which the records have been age-corrected. This can not be done directly, without knowing the age-correction factors which we seek to establish; whatever they are, the following is true:

$$\text{Age-corrected herd average} = \text{actual herd average} + \text{corrections for age.} \quad (1)$$

This statement does not imply additive factors for, even with multiplicative factors, the actual correction to a cow's record can be expressed as an additive correction and used in this equation. To define the corrections for age in this equation, the young cows were divided into four age groups, as shown in Table 1. The variables studied for these groups were the differences between the mature cow average and each of the young cow averages, for a herd-year-season; namely,

$$\text{Age difference} = \text{mature cow average} - \text{young cow group average.} \quad (2)$$

The data came from the files of the Dairy Record Processing Center of the Animal Husbandry Department at Cornell University. The cows in each herd had been divided into two seasonal groups, those calving in March through August being the spring freshening group, and those calving in September through February being the fall freshening group. Within each herd, for each seasonal group in each year, the mature cow averages and the young cow group averages were differenced as in Equation (2), to obtain the age-difference variable. This age difference can be thought of as an age correction, because it represents the correction one would wish to make to a young cow's record, to bring it up to the mature cow level of that herd in the same year and season of freshening. Since the data for estimating the correction by this difference

TABLE 1

Definition of age groups

Age groups	Age in months at freshening
Young cows, Group 1	19 – 27
Young cows, Group 2	28 – 34
Young cows, Group 3	35 – 40
Young cows, Group 4	45 – 59
Mature cows	60 – 114
Excluded from analysis	18 and less
	115 and above

is so limited within a single herd, regressions were set up of the age-difference variable on the age-corrected herd average, namely

$$\text{Age difference} = \alpha + \beta\ (\text{age-corrected herd average}) + \text{error}, \tag{3}$$

one such regression for each of the four young-cow age groups. These regressions allow a simplification of Equation (1) for the age-corrected herd average, as is now shown.

Let N_i be the number of cows in the ith herd, n_{it} and y_{it} be the number of cows and their mean yield, in Age Group t in the ith herd, t taking Values 1, 2, 3, 4, and m, for mature; and let x_i be the mean yield of all cows in the herd. Then, if h_i is the age-corrected herd average of the ith herd, Equation (1) is

$$h_i = x_i + \text{age corrections}, \tag{4}$$

and by (2) the age differences are

$$z_{it} = y_{im} - y_{it}, \text{ for } t = 1, 2, 3, \text{ and } 4. \tag{5}$$

The regressions in (3) are then

$$E(z_{it}) = \alpha_t + \beta_t h_i, \text{ for } t = 1, 2, 3, \text{ and } 4, \tag{6}$$

where E denotes expectation over the population of herds.

If these regressions were known exactly, the age-correction for a cow in the tth young-cow age group would be $\alpha_t + \beta_t h_i$ and with n_{it} such cows in the ith herd $n_{it}(\alpha_t + \beta_t h_i)$ would be the correction due to these cows to be added to the total yield of the herd in obtaining the age-corrected herd average. Substituting these expressions for $t = 1$, 2, 3, and 4 into (4), gives a value for h_i which simplifies to

$$h_i = \frac{N_i x_i + \sum_t n_{it}\alpha_t}{N_i - \sum_t n_{it}\beta_t}. \tag{7}$$

When this expression is substituted in Equations (6), the regressions are entirely in terms of the unknown parameters, the four α's and four β's which have to be estimated from the observable variables z_{it} ($t = 1$, 2, 3, and 4) and x_i.

The customary least-squares procedure of estimation applied to this situation leads to equations of the fourth degree in the unknowns which have no simple solution. No doubt a solution could be found by an iterative process, but as iteration can be used in the equation for h_i, a more straightforward method was available. The regressions in (6) are in terms of h_i which is initially unknown, but for any set of values of the α's and β's h_i is known by Equation (7). By assuming these as zero, a first approximation to h_i is, therefore, x_i, the actual herd average. Using this in (6), the first estimates of the α's and β's were obtained by the usual least-squares analysis, applied to each regression separately. These first estimates were then substituted in (7) to obtain a second approximation to h_i which, in turn, was used to re-estimate the regressions in (6), the process being continued until the values of the β's were the same on two successive occasions. These values were then considered to be the estimates of the regressions. The linear relationships shown in (4), (5), and (6) ensure

that such a procedure does have a unique solution. No consideration was made for the variables x_i, y_{it}, and z_{it} being averages, with differing numbers of records in them and, hence, different sampling variances. A weighted regression takes account of these, but since the iterative procedure outlined above involved less computational problems, it was used, basing the analysis on a large amount of data. Each round of the iteration entailed only standard linear regression analyses, and sampling variances of the final estimates were obtained by minor adaptations of standard results, using the final approximations to the h_i as the independent variables [see Searle (18)].

A simpler approach to the problem would appear to be that of using the regressions of the young cow averages y_{it} ($t = 1, 2, 3$, and 4) on the herd average x_i. Using a linear mathematical model involving age, herd, and cow-within-herd effects, it can be shown [Searle (18)] that y_{it} is correlated in a sampling sense with x_i, whereas z_{it} is not, so that the regressions using z_{it} are more appropriate.

DATA AND RESULTS

The data consisted of 20,532 Holstein records in 749 herds in New York State in the four seasons fall, 1950, through spring, 1952. They were obtained by choosing at random a range of herd numbers from the herd file of the New York Dairy Herd Improvement Cooperative's records, for the fall season of 1950. The same range of herd numbers was used in the three other seasons, and from all these data the herds having some cows in all four seasons were sorted out. These were divided in half and analyzed as two separate groups. A third group was also analyzed, being a larger sample of smaller herds that did not have cows in all four seasons.

In each group the four regressions were estimated simultaneously by the iterative procedure, using an IBM 650 electronic computer. In all cases, the estimates had settled down by the fourth round, that is to say, the estimated β's at the fifth round were the same as those at the fourth. Adaptations of standard tests of hypotheses showed no significant differences between the three groups, as was to be expected, since they are all samples of the same population. The results are, therefore, shown for the three groups pooled together.

Table 2 shows the division of the herds according to the number of year-seasons in which they had cows in milk. In performing the calculations, it was found that 231 of these 2,115 herd-year-season groups had only mature cows in them, so that these contributed nothing to the estimation. Not all of the remain-

TABLE 2

Number of herds and herd-year-seasons

Number of year-seasons in which a herd is included	Number of herds	Number of herd-year-seasons
1	182	182
2	126	252
3	83	249
4	358	1,432
Total	749	2,115

ing 1,884 herd-year-season groups had cows in every age group. Therefore, the number of herd-year-seasons on which each regression estimate is based differs from age-group to age-group; these numbers are shown in Table 3. The mean age difference, and the number of records of each age group, also are shown.

The study is confined to records of Holstein-Friesian cows, both grade and purebred, and the analysis was made on butterfat yield, excluding yields less than 100 lb. Table 4 shows the mean herd average butterfat according to season and year, the over-all mean herd average being 397 lb. butterfat. It is noticeable

TABLE 3
Summary of data by age group

Age group	No. of herd-year-seasons from which regression is estimated	Mean difference from mature cows	No. of records in age group
		(lb. butter fat)	
1	935	113	2,276
2	1,080	90	2,706
3	1,383	65	3,564
4	1,420	23	3,981
Mature	.....	.....	8,005
Total number of records in the study			20,532

TABLE 4
Mean herd average according to year and season

Year and season	Mean herd average
	(actual lb. fat)
Fall, 1950	399
Spring, 1951	394
Fall, 1951	397
Spring, 1952	397
All seasons	397

that the difference often observed between the fall and spring seasons is not apparent here. There seems to be no explanation for this, except to say that it has been observed in other samples of data used for analysis purposes, namely Henderson (9).

The estimates of the regression parameters, pooled from the three groups of data, are shown in Table 5. The standard error of the estimated slopes was estimated as .032; tests of hypotheses indicate that each of these slopes is significantly different from zero. The standard error of the difference between the slopes of two lines was estimated as .04. Therefore, one could combine the regression slopes of the first three age groups, but there seems to be no real advantage in doing this, and further work was based on the estimates as shown in Table 5.

These estimates substituted into Equation (7) give the following expression for the age-corrected herd average:

$$h_i = \frac{N_i x_i + 8n_{i1} - 19n_{i2} - 18n_{i3} - 8n_{i4}}{N_i - (.24n_{i1} + .25n_{i2} + .19n_{i3} + .07n_{i4})} \quad (8)$$

and for any herd whose age-corrected herd average by this formula is h, the age corrections are as shown in Table 5.

It is interesting to look at the differences between these age corrections for different herd levels, i.e., for different values of h. Most herds in New York State have a mature equivalent average within the range of 300–600 lb. of butterfat, so discussion will be confined to this range of values. The mean value of the final approximations to the h_i in these data was 440 lb., and the variance among them was approximately 4,000. Using .0015 for the variance of the difference between two estimated β's, it can be shown that the variance of the difference between two estimated age corrections based on the same h is

$$[4{,}000 + (h - 440)^2]\ (.0015).$$

The age-correction factors for various values of h and the standard error of the difference between pairs of them are shown in Table 6.

TABLE 5

Estimated age corrections for a herd having an age-corrected herd average h.

Age group	Age correction [a]
1	$8 + .24h$
2	$-19 + .25h$
3	$-18 + .19h$
4	$-8 + .07h$

[a] Estimates of the regression lines in Equations (6).

TABLE 6

Estimated age-correction factors for herds of different production levels, as indicated by their age-corrected herd average; and the estimated standard error of differences between these estimates for each herd level

Age group	Herd level h			
	300	400	500	600
1	80	104	128	152
2	56	81	106	131
3	39	58	77	96
4	13	20	27	34
Standard error of a difference	6	2	3	6

The usual tests of hypothesis applicable to the values in Table 6 show that within each herd level there is a significant difference between the correction factors of any pair of age groups.

DISCUSSION

Two main results. (i) The estimated regression lines are shown in Figure 1. These lines are the regressions of the age corrections on the age-corrected herd average, and for each group the slope is significantly different from zero. This means that the age-correction factors developed here are significantly different from one herd level to another, so that for cows of the same age those in herds

having a high level of production should receive larger additive age corrections than those in herds of a low level of production. For example, cows in Age Group 1 should receive 80 lb. if in 300-lb. herds (Table 6), but they should receive 152 if in 600-lb. herds. Likewise, cows in Age Group 4 should receive

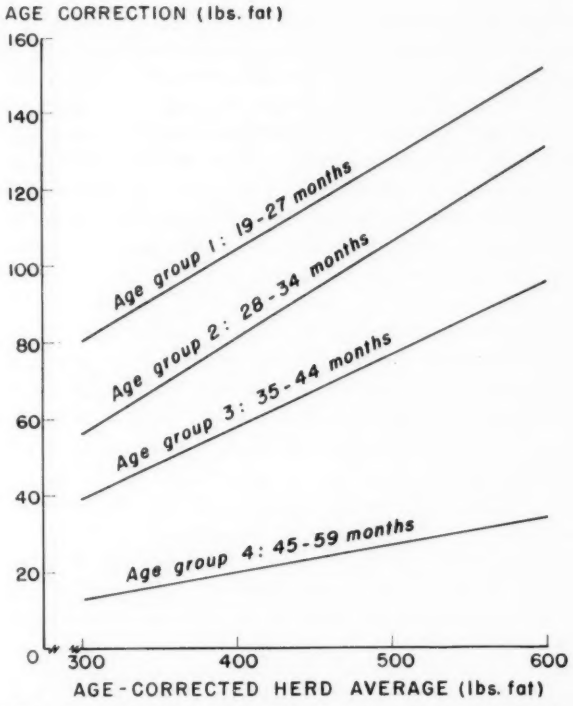

FIG. 1. Estimated age-correction factors based on the age-corrected herd average for cows in different age groups.

age corrections of 13 and 34 lb. when in herds of 300- and 600-lb. production level, respectively. This result is contrary to Hickman and Henderson (11), but it agrees with trends noted in the New Zealand data of Castle (2).

(ii) The results indicated in Table 6, that there are significant differences between the age groups, are in complete accord with all previous work, that cows of different ages differ in production.

Corrections effective in different ways. The age corrections here are not the same as the multiplicative ones or the additive ones. When additive factors are used, the same quantity is added to the records of all young cows of the same age, independent of their own production and of the production of the herd they are in. This means that the young cows which are inherently poor producers have the same amount added to their records as those which are good producers.

If this makes a true correction for age, it implies that age affects production of all cows in the same way, no matter what their real producing ability is. This means that the difference between what a cow produces when it is young and what it would have produced in the same environment had it been but older, is the same for cows of all real producing abilities. Because production environments can not be exactly replicated, it is impossible to observe such differences; cows which are 2-yr.-olds now can not suddenly become mature cows and produce again in the same environment. Therefore, the only indications of this difference are those seen between productions of the same cows at different ages, which include differences between environments, as well as other effects. These seem to indicate that the situation under which additive factors would be correct does not hold true, in that cows which have a high production when young have different increases to maturity from those which are low producers when young. Johansson and Hansson (13) and Ward and Campbell (19) both indicated that differences between production records at mature ages and at young ages of the same cow, were smaller for cows whose first records were large, than for those whose first records were low. From this, they argue that multiplicative factors over-correct the records of the higher-producing young cow and under-correct those of the low-producing young cow, and so conclude that additive factors are preferable. This would be so, except that what they have observed is not comparable with the purpose of age-correction factors, which is not to predict future records.

Successive records of the same cow tend to be regressed toward the mean of the herd. Therefore, if a cow makes an above-average record when young, it is probable that it will not show such a big increase in its mature records as a cow making a below-average record when young. If the environment in which its record as a young cow was made was such that it was a high record then, had that cow been mature, might not that same environment have led to an even higher record than it did actually make as a mature cow? Likewise, for the low-producing cow: if the environment in which a cow's record was made when it was young was such that it was a low record, then, had that cow been mature, might not that same environment have led to a yield lower than it did actually make as a mature cow, 3 or 4 yr. later? If so, multiplicative factors, by giving a correction to a record in proportion to the size of the record, take some account of this situation. However, in doing so, the environment in which the young-cow record was made is then taken into account only as it is reflected by the record itself. This is appropriate for the permanent environmental effects peculiar to the cow, but it is not a reliable indication of the wider environmental factors affecting production concerned with herd management. Thus, if there is an interaction between herd environment and the effects of age on production, basing age-correction factors on the record alone takes no account of it, whereas age corrections based on herd level would appear to do so.

Cows of the same age in the same herd will receive different additive age corrections when multiplicative factors are used. However, to the extent that rate of maturity—which is what age corrections are basically designed for—

is governed by herd environment and herd management, it would be more appropriate to give cows of the same age in the same herd the same age correction. Whereas the multiplicative factors assess environment on the basis of the cow's record, the factors developed here assess it on the herd average; probably neither situation is correct, and the true factors lie somewhere between these two. Basically, the question is whether herd mates of the same age should receive different age corrections (presumably utilizing the actual record, as with multiplicative factors), or whether they should receive the same age corrections (based on herd level, as developed here).

Comparison with New York multiplicative factors. The age correction developed here, for a cow in a herd with age-corrected herd average h is in the form $z = \alpha + \beta h$; if this is effective, it will bring the cow's actual record up to a maturity equivalent; but h represents the mature equivalent herd level, so that for the average cow $h - z$ is its actual production and h its mature production. Therefore, since

$$h = (h - z)\frac{h}{h - z}$$

identically, the multiplicative factor for converting $h - z$ to h is

$$\frac{h}{h - z} = \frac{1}{1 - \alpha - \beta/h}.$$

For the four age groups and the limits of 300–600 lb. butterfat for h being considered here, these derived multiplicative factors are shown in Table 7, in comparison to the factors used in New York State.

TABLE 7
Direct comparison with multiplicative factors

Age group		Factors of present study converted to multiplicative basis		Mean multiplicative factor used in New York State
		Age-corrected herd average		
Range	Mean	300	600	
(age in months)				
19–27	24	1.36	1.34	1.35
28–34	31	1.23	1.28	1.24
35–44	39	1.15	1.19	1.18
45–59	52	1.05	1.06	1.06

In this form, the new factors are very similar to those being used at present. If this means that the two sets of factors are equivalent, it would indicate, judged on the basis by which the factors of this study were developed, that the multiplicative factors satisfactorily take account of herd differences in the age corrections that should be used. A conclusion such as this would be most satisfactory, but it can not be made decisively on this comparison alone, based as it is on a situation pertaining only to average records.

The problem of judging between two sets of age-correction factors by comparing the efficiencies with which they achieve their purpose, presupposes that

this efficiency can be measured satisfactorily. Possible methods of doing this are at present under consideration and will be reported upon shortly.

Method of application. The age-correction factors for any herd are obtained by using Expression (8) for the age-corrected herd average, and using this in Table 5 for the age-correction factors. The procedure will be demonstrated by a small hypothetical example.

Suppose a herd has seven cows, one being in Age Group 1, three in Age Group 2, and three being mature cows, with butterfat records as follows:

Age Group	Actual record (lb. fat)
1	300
2	400, 470, 463
Mature	479, 490, 500
Total	3,052

The first term in the numerator of (9) is the total of the actual herd production, because it is the product of N_i, the number of cows in the herd, and x_i, the actual herd average, so that (8) can be written as

$$\text{Age-corrected herd average} = \frac{\text{Actual herd total} + \begin{cases} 8 \times \text{No. of cows in Age Group 1} \\ -19 \times \text{No. of cows in Age Group 2} \\ -18 \times \text{No. of cows in age Group 3} \\ -\ 8 \times \text{No. of cows in Age Group 4} \end{cases}}{\text{No. of cows in the herd} - \begin{cases} .24 \times \text{No. of cows in Age Group 1} \\ .25 \times \text{No. of cows in Age Group 2} \\ .19 \times \text{No. of cows in Age Group 3} \\ .07 \times \text{No. of cows in Age Group 4} \end{cases}}$$

Therefore, in the example

$$h = \frac{3{,}052 + (8 \times 1 - 19 \times 3)}{7 - (.24 \times 1 + .25 \times 3)} = \frac{3{,}003}{6.01} = 500.$$

The results in Table 6 then give the age corrections as

Age Group	Age correction
1	$8 + .24(500) = 128$
2	$-19 + .25(500) = 106$

Adding these to the actual records gives the age-corrected records as follows:

Age Group	Age correction added	Age-corrected records
1	128	428
2	106	506, 526, 569
Mature	....	479, 490, 500
Total		3,498

Thus, the mean of the age-corrected records is $3{,}498/7 = 500$, which is the same as the age-corrected herd average calculated by the formula. This is true in all cases, that the age-corrected herd average calculated from the formula and used for obtaining the age-correction factors is exactly the same as the average of those records after they have been so corrected. This is a direct consequence of the method and, naturally, a desired requirement.

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EFFECT OF THE DIETARY RATIO OF HAY TO CONCENTRATE ON MILK PRODUCTION, RATION DIGESTIBILITY, AND URINARY ENERGY LOSSES

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SUMMARY

Lactation and balance studies were carried out with 12 Holstein cows in a randomized complete block design. Twenty, forty, or sixty per cent of the estimated net energy (ENE) was supplied in the form of hay; the remainder in the form of concentrate. The level of ENE intake above maintenance was held constant. FCM production was approximately the same for all groups. A significant and rectilinear increase in pounds of FCM per therm of digestible energy consumed above maintenance was observed as the proportion of concentrates in the ration increased. Digestibility data are presented. Urinary energy losses were closely correlated ($r = 0.96$) with urinary nitrogen losses.

It is generally recognized that the digestible energy (DE) and total digestible nutrient (TDN) systems overevaluate roughages relative to concentrates for productive purposes. This was observed many years ago by Wolff *et al.* (20), and repeatedly has been demonstrated since that time (9, 10, 11, 16, 17, 18). The fundamental reason for it, however, remains controversial even today (18).

If the idea is accepted, that the explanation lies in differences in productive energy input, then differences must exist in the magnitude of methane, urinary, or heat losses. There appears to be little basis for the belief that methane losses are larger for roughages than for concentrates (15). Urinary energy losses have not been studied extensively in milk production trials, but in general are believed to be small (15).

Recent calorimetric studies (1–4) with sheep suggest the hypothesis that the efficiency with which DE (or TDN) is utilized for milk production is related to the relative proportions of the volatile fatty acids (VFA) produced in the rumen fermentation.

The present studies were undertaken to determine the relative efficiency with which DE was utilized for milk production and the magnitude of urinary energy losses at three different ratios of hay to concentrate, and to test the above hypothesis. This paper deals with milk production and fecal and urinary energy losses. Production efficiency as related to rumen VFA ratios will be the subject of another publication.

EXPERIMENTAL PROCEDURE

Animals and rations. Twelve purebred Holstein cows, which had demonstrated a productive ability of more than 9,000 lb. of milk per lactation, were used in these experiments. On the basis of age and previous performance, they

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were grouped in trios and assigned to a randomized complete block design with three treatments. Within 1 wk. after calving, each animal was placed on the experiment, which consisted of a 4-wk. preliminary period, a 2-wk. adjustment period, a 14-wk. experimental period, and a 2-wk. postexperimental period.

The feeds used were second-cutting alfalfa light grass mixed hay (U. S. No. 2) and a concentrate mixture containing (in pounds) ground yellow corn, 210; ground oats, 200; wheat bran, 100; corn distillers dried grains, 400; cane molasses, 70; dicalcium phosphate, 10, and salt, 10. The mixture was fortified with 1 g. of cobalt sulfate per 1,000 lb. Additional salt was available on a free-choice basis. Two of the replicates also received (in connection with another experiment) a vitamin-mineral supplement. The average composition of the feeds is presented (Table 1). For purposes of determining estimated net energy (ENE) intakes, and making hay-concentrate substitutions, the ENE value of the concentrate was calculated (13) to be 78.1 therms per 100 lb.; that of the hay was estimated at 40.1 therms.

During the preliminary period, hay was fed to capacity with a concentrate allowance of approximately 2 lb. for each 3 lb. of hay consumed daily. At the end of the preliminary period each cow was adjusted, over a period not exceeding 1 wk., to its respective experimental ration, which was then fed unchanged throughout the experimental period. The experimental rations supplied 60, 40, or 20% of the ENE intake in the form of hay. Each cow was offered sufficient ENE to support maintenance (based on weight at the end of the preliminary period) and the daily production of 45 lb. of 4% fat-corrected milk (FCM), using Morrison's standards (upper levels).

In the hope of obtaining data on body weight changes which would be relatively free of changes in gastro-intestinal "fill," the animals were returned for a 2-wk. postexperimental period to a full-feeding program similar to that in the preliminary period. Weights were then obtained for comparison with the weights at the end of the preliminary period.

Throughout these studies all feeds and refusals and milk yield were recorded to the nearest 0.1 lb. Feeding was done twice daily in equal parts. The cows were milked twice daily and milk samples for butterfat analysis were obtained from four consecutive milkings weekly. Body weights were obtained on three consecutive days at the ends of the preliminary, experimental, and postexperimental periods. Weekly weights (one day) were also recorded during the experimental period.

TABLE 1

Mean proximate composition and heats of combustion of feeds with their standard errors

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Ash	Heat of combustion
	(%)						*(kcal/kg)*
Hay	88.1	15.9	1.8	29.4	34.0	7.1	4,070
	0.3	0.3	0.1	0.8	0.5	0.2	17
Concentrate	87.4	16.9	5.5	9.0	51.0	5.0	4,213
	0.2	0.1	0.2	0.3	0.5	0.1	30

Balance studies. During the experimental period, each cow was subjected to a seven-day balance trial in which the digestibility of the ration was determined by conventional methods. Urinary energy and nitrogen losses also were determined during these trials.

Analysis. Feed samples were, with a few exceptions, taken weekly and composited for monthly analysis. During the balance studies, core samples of hay were obtained from at least one-half of the bales used during each collection period, and concentrate samples were taken daily.

All feed and fecal samples were analyzed for moisture, ether extract, crude protein, crude fiber, and ash, using AOAC (5) methods with modifications as employed in this laboratory, and heats of combustion were determined in a Parr adiabatic oxygen bomb calorimeter. The urine, which was preserved with a small amount of sodium fluoride, was analyzed for nitrogen, and the heat of combustion was determined using the technique of Eriksson (8), with modifications.

RESULTS

Feed consumption, milk production, and body weight changes. A summary of the mean daily feed consumption, FCM production, and body weight changes is presented (Table 2). All cows consumed their rations essentially as planned.

TABLE 2

Average daily feed consumption and milk production, average body weight changes, and efficiency of energy utilization

Criterion	ENE from roughage *(%)*		
	60	40	20
Hay consumption *(lb.)*	32.3	21.9	10.6
Concentrate consumption *(lb.)*	11.2	17.0	21.8
ENE consumed *(therms)*	21.7	22.1	21.3
ENE consumed above maintenance *(therms)*	13.4	13.5	13.5
DE consumed above maintenance[a] *(therms)*	30.7	26.8	23.2
FCM production *(lb.)*	44.3	46.0	45.8
Body weight change *(lb.)*	−18	−25	−23
FCM/therm ENE above maintenance	3.3	3.4	3.4
FCM/therm DE above maintenance	1.4	1.7	2.0

[a] DE required for maintenance was obtained by conversion of Morrison's (13) standards, using the factor 2,150 Kcal DE/lb TDN as determined in 22 trials with these feeds. Maintenance requirements were based in all cases on weight at the end of the preliminary period.

Since the cows on the highest ratio of hay to concentrate were fed at close to capacity, hay refusals in this group were, on the average, slightly higher than in the other groups; however, on no ration did refusals average more than 1.2% of that fed.

Average daily production of FCM during the 6 wk. preceding the experimental period was uniform (60.0, 58.0, 59.1 lb.) among the three treatment groups. It can be seen from the table that the average production during the experimental period was also very similar among groups, as expected. One of the purposes in using the ENE system for rationing in this study was to eliminate plane of nutrition as a variable. This objective appears to have been closely

approached. While efficiency in the utilization of ENE for milk production was almost constant, regardless of the ratio of hay to concentrate, there was a significant ($P < 0.05$) and rectilinear increase in the efficiency with which DE was converted to milk as the proportion of concentrate in the ration increased (Table 2).

Mean body weight changes, when freed of any marked effect of "fill," were not appreciably different among groups. This was to be expected, since all cows were fed at approximately the same level of productive energy. There was considerable variation within groups, however, most of which appeared to be associated with replication. Without exception on any treatment, the cows in the two replicates which had been selected on the basis of higher previous production, and which demonstrated the highest efficiency in these experiments, lost appreciably more body weight than the others.

Digestibility. Mean apparent digestion coefficients for dry matter, crude protein, ether extract, nitrogen-free extract, crude fiber and gross energy, and the TDN values for each ration, are presented (Table 3). As the proportion of

TABLE 3

Mean apparent digestion coefficients and TDN values

ENE from roughage	Dry matter	Crude protein	Ether extract	N-free extract	Crude fiber	Gross energy	TDN (DM basis)
				(%)			
60	65.0	75.2	58.4	75.5	46.6	65.1	63.6
40	68.2	75.4	72.4	77.8	47.9	68.2	68.4
20	71.9	75.8	78.4	80.0	48.6	72.2	73.2

concentrate in the ration increased, the apparent digestibility of the dry matter, ether extract, nitrogen-free extract, and gross energy increased, as did the TDN values. Differences among rations in these criteria were significant ($P < 0.01$). Crude fiber and crude protein digestibilities were not significantly affected by the ratio of hay to concentrate.

Urinary losses. The urine nitrogen and energy losses during the seven-day balance trials are summarized in Table 4. It is evident from these data that the energy losses were closely related to the nitrogen losses. Similar data were available from an additional eight trials in which the ration consisted only of the hay used in these studies. In view of the high correlation ($r = 0.96$) between these variables, a regression equation ($Y = 23.8 + 125.0\ X$) relating energy

TABLE 4

Mean urine nitrogen and energy losses

ENE from roughage	Total urine	Total nitrogen	Total energy	Calorie-nitrogen ratio	Total energy
(%)	*(kg.)*	*(g.)*	*(kcal.)*	*(kcal/g)*	*(% of DE)*
60	105.1	1,548	22,466	14.5	6.1
40	103.2	1,420	20,453	14.4	5.9
20	82.5	1,022	14,506	14.2	4.7

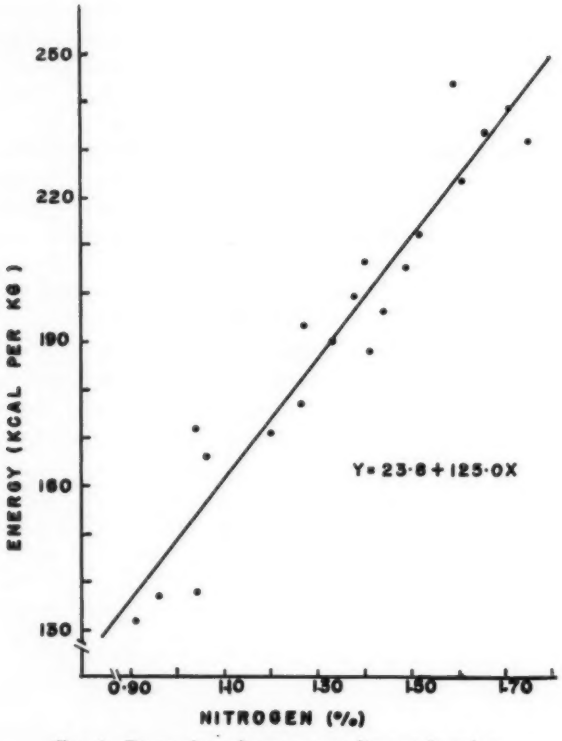

FIG. 1. Regression of energy on nitrogen in urine.

per kilogram *(Y)* to nitrogen content *(X)* was fitted to the individual data from the 20 trials (Figure 1). The standard deviation from regression was 9.8.

Although a greater percentage of the DE consumed was lost as urine with increasing proportions of roughage in the ration, this appears to be explained in terms of an increasing nitrogen intake in relation to requirements. Since it was desirable in this study that the hay and concentrate have approximately the same content of crude protein, the method of rationing used resulted in a greater total crude protein intake as roughage supplied an increasing proportion of the ENE. The digestible protein intake exceeded requirements in all groups.

DISCUSSION

The results of this experiment support the general contention that DE and TDN overevaluate roughages relative to concentrates for milk production. It has been suggested that this discrepancy is due to a difference in productive energy input (10, 11). The data from these studies lend support to this interpretation.

The use of apparent digestion coefficients which were directly and conven-

tionally determined during the lactation study, eliminates the possible inclusion of any difference attributable to nonadditive digestibility of the roughage and concentrate.

While the urinary energy losses were greater with increasing proportions of roughage, these differences would explain only a small part of the observed differences in efficiency. Furthermore, urinary energy losses were closely related to urinary nitrogen losses. In practice, nitrogen intake and excretion are frequently greater on rations with higher proportions of concentrate. Smaller urinary energy losses, therefore, could not offer a logical explanation for their greater efficiency.

The concentrate used in this study had a higher content of ether extract than the roughage. Using the average chemical composition of the feeds and average digestion coefficients, the mean daily digestible ether extract intakes for the three groups were calculated to be 0.70, 0.96, and 1.09 lb. The possibility that this influenced the efficiency with which the available energy was utilized can not be disregarded in the light of the studies (12) demonstrating that the fat in dairy cattle rations is worth more than its TDN value would indicate; nor can the possible effect of the differences in protein intake be overlooked. However, the greatest difference in nutrient intake among treatment groups was in crude fiber, the cows on the highest ratio of roughage to concentrate consuming daily approximately 10.5 lb., and those on the lowest ratio consuming approximately 5.1 lb.

The heat increment of carbohydrate in the ruminant includes a part which may be attributed to the physical work associated with the intake and digestion of feed. Thus, a part of the observed differences in efficiency may be attributed to differences in dry matter intake. However, more attention has recently been focused on that part of the heat increment arising from the wasteful metabolism of the rumen fatty acids. Armstrong and associates (2, 4) have demonstrated the lower efficiency with which acetic acid, when compared with propionic and butyric acids, is utilized by fattening sheep. Since the ratio of acetate to propionate in rumen contents has been shown to be lower on rations containing large proportions of concentrate (6, 7, 14, 19), a relationship between the efficiency with which DE is utilized for milk production, and the relative proportions of the rumen VFA, would appear to be plausible.

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RELATIONSHIP OF MILK PRODUCTION EFFICIENCY TO THE RELATIVE PROPORTIONS OF THE RUMEN VOLATILE FATTY ACIDS

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SUMMARY

The molar composition of the rumen VFA was studied at three dietary ratios of hay to concentrate in 12 dairy cows used in a lactation-balance experiment previously reported. As the proportion of concentrate in the ration increased, the relative proportion of acetic acid decreased, whereas that of propionic and butyric acids increased. Correlation analyses indicated that the efficiency with which digestible energy was converted to FCM was highly correlated with the relative proportion of propionic acid in the rumen VFA and with the ratio of acetate to propionate, as well as with the crude fiber content of the ration. The plausibility and some of the implications of a causal relationship are discussed.

In a previous publication (7), data on the effect of three ratios of hay to concentrate on milk production and fecal and urinary energy losses were presented and discussed. Reports by Armstrong and associates (1, 2), indicating that the heat increment of acetic acid is of considerably greater magnitude than that of propionic and butyric acids when administered to sheep, led the authors to investigate the relative distribution of the rumen volatile fatty acids (VFA) in the cows used in the lactation study. These data and their relationship to the efficiency with which the digestible energy (DE) was utilized for milk production are the subject of the present paper.

EXPERIMENTAL PROCEDURE

Following the balance trials (7), and again approximately 4–6 wk. later, samples of rumen contents (approximately 200 ml.) were obtained from each cow by stomach-tube, using a vacuum pump. All samples were collected 4–4.5 hr. following the afternoon feeding. The samples were immediately taken to the laboratory and strained through cheese cloth. The resulting rumen liquor was preserved by adding mercuric chloride (1 ml. saturated solution per 50 ml. rumen liquor) and refrigerating at approximately 40° F. in air-tight bottles, until analyzed.

Acetic, propionic, butyric, and valeric acids were separated chromatographically by the procedure of Wiseman and Irvin (17), as adapted for rumen liquor by Stewart (14). The valeric acid fraction contained small amounts of higher acids. The relative concentrations of the acids were expressed as moles per 100 moles of volatile fatty acids (VFA). Results of the two samples available from each cow were averaged. No attempt was made to determine possible dif-

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ferences among rations in the absolute concentration of VFA, since it was felt that the stomach-tube sampling technique would invalidate the results due to possible and variable salivary dilution. The ratio of acetate to propionate (A/P ratio) as used in this study is the molecular ratio.

Milk production efficiency was calculated (using appropriate heats of combustion for the feeds and average digestion coefficients for the rations) as either pounds of FCM per therm of DE consumed (gross efficiency) or as pounds FCM per therm of DE consumed over and above maintenance requirements (net efficiency).

Standard statistical methods as outlined by Snedecor (13) were used in analyzing the data.

RESULTS

VFA ratios. The mean molar composition of the rumen VFA for each ration is presented (Table 1). As concentrate made up an increasing proportion of the ration, the relative proportion of acetic acid decreased, with concomitant increases in propionic and butyric acids. Differences among rations were statistically significant ($P < 0.05$) in each of these. The relative proportion of valeric acid was not significantly affected.

TABLE 1
Mean molar composition of rumen volatile fatty acids

ENE from roughage	Valeric	Butyric	Propionic	Acetic
(%)	*(moles per 100 moles of VFA)*			
60	2.9	11.8	18.7	66.6
40	2.9	13.0	19.4	64.7
20	2.7	15.4	21.4	60.5

Production efficiency as related to rumen VFA. To study possible interrelationships between production efficiency and the relative proportions of the rumen VFA, correlation analyses were conducted. The correlation coefficients between a number of the variables studied are presented (Table 2). The relative concentration of propionic acid in the rumen liquor and the gross or net production effi-

TABLE 2
Correlation coefficients among variables studied

Variables	Correlation coefficient (r)
Gross production efficiency and molar per cent propionic acid	0.88
Net production efficiency and molar per cent propionic acid	0.83
Gross production efficiency and A/P ratio	−0.83
Net production efficiency and A/P ratio	−0.80
Net production efficiency and molar per cent acetic acid	−0.76
Net production efficiency and molar per cent butyric acid	0.61
Net production efficiency and crude fiber content of ration	−0.85
Crude fiber content of ration and molar per cent acetic acid	0.89
Crude fiber content of ration and molar per cent propionic acid	−0.84

ciency of the individual cows in this study were found to be highly correlated. Correlations (negative) of similar magnitude existed between the A/P ratio and gross and net efficiencies. Since the molar percentage of propionic acid appeared to be the variable most closely associated with production efficiency, regression equations relating gross efficiency and net efficiency *(Y)* to the molar percentage of propionic acid *(X)* were fitted to the individual data, which are presented graphically (Figures 1 and 2). The equation for gross efficiency is $Y = -0.287 + 0.0615\,X$, with a standard deviation from regression of 0.057, while that for net efficiency is $Y = -1.040 + 0.1387\,X$, with a standard deviation from regression of 0.159. Net efficiency was less closely correlated with the molar percentage of acetic or butyric acids.

Blaxter's (4) discussion of the effect of the fiber content of the ration on rumen VFA ratios, and on the utilization of energy, prompted a study of these interrelationships. The molar proportions of acetic and propionic acids were found to be closely related to the average crude fiber content of the ration, and net efficiency likewise was highly correlated with the average crude fiber content of the ration.

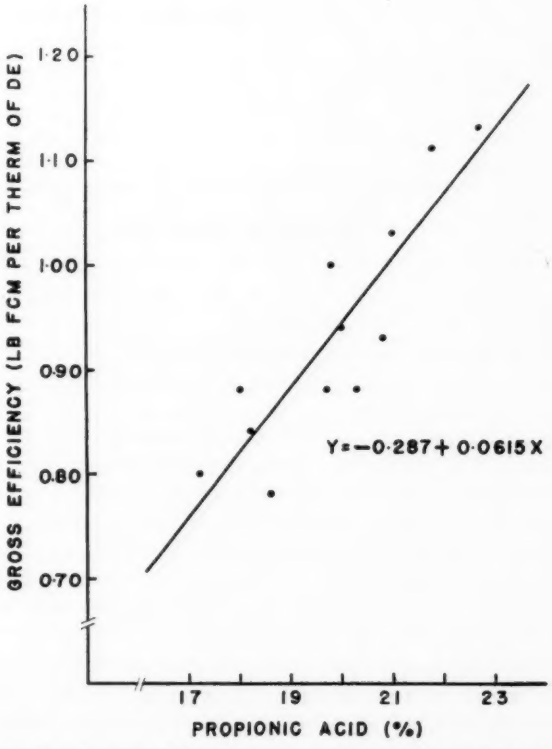

FIG. 1. Regression of gross efficiency on the molar percentage of propionic acid in the rumen volatile fatty acids.

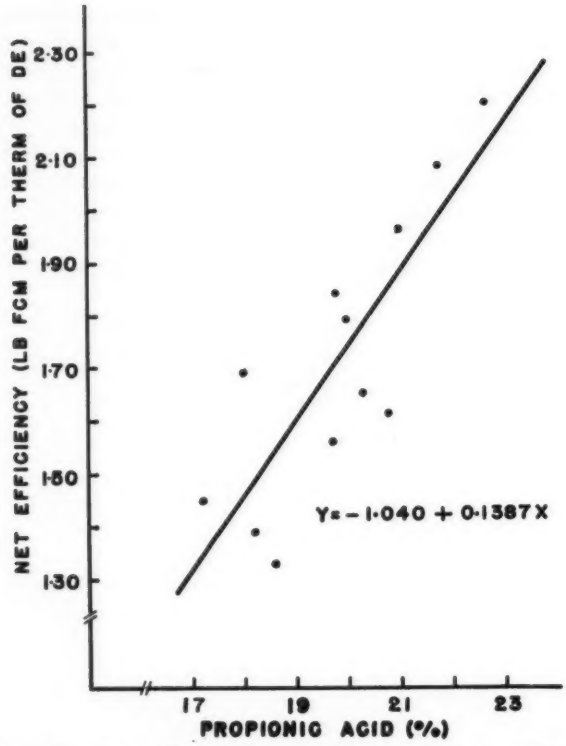

FIG. 2. Regression of net efficiency on the molar percentage of propionic acid in the rumen volatile fatty acids.

DISCUSSION

While these findings in no way establish a causal relationship between the relative proportions of the individual acids in the rumen VFA, on the one hand, and efficiency of energy utilization on the other, they strongly support the hypothesis under test. In the light of the calorimetric experiments already discussed, a meaningful relationship seems not only possible but probable.

The VFA data are in general agreement with previously published results of others (3, 5, 12, 16). It is recognized that the differences among rations may not be true reflections of the absolute differences in production. However, there is recent evidence supporting the idea that the relative proportions of the VFA as measured in rumen liquor may be a reasonably accurate estimate of the proportions in which they are absorbed (6, 10, 15).

The crude fiber–VFA and crude fiber–efficiency relationships are in agreement with observations cited and discussed by Blaxter (4). The results also tend to confirm those of Nordfeldt *et al.* (11), who reported decreases in milk production with increasing crude fiber in the ration, when TDN intake was held constant.

A closer correlation between the molar proportion of acetic acid and production efficiency might have been expected, in view of the finding of Armstrong and associates (1, 2) that the heat increment of acetic acid was much larger than that for propionic and butyric acids. On the other hand, there is no a priori reason to assume that the efficiency with which these individual metabolites are utilized for milk production is identical to that with which they are utilized for fattening. There is, indeed, evidence (9) that this is not the case.

A causal relationship might explain, in part at least, many of the "associative" metabolic effects of feeds, such as those observed in the calorimetric experiments of Forbes *et al.* (8). As suggested by Blaxter (4), it would offer a reasonable explanation for differences in the productive value of the TDN from different feeds. Its fuller understanding might permit significant improvements in the efficiency with which the ruminant utilizes the energy in roughages.

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EFFECT OF CORN STARCH ON DIGESTIBILITY OF ALFALFA HAY

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SUMMARY

Digestibility studies have been conducted on dairy cattle to investigate the effect of a readily digestible carbohydrate on the digestibility of alfalfa hay. Digestion trials of five or six days' duration were carried out by the total collection method. When a 20-day preliminary period was allowed, the added carbohydrate had little, if any, effect on alfalfa digestibility. When no time was granted for adjustment to the starch addition, a highly significant depression of NFE, and significant decreases in protein and dry matter digestibilities, were observed. Evidence of other workers having a bearing on the relationship between preliminary periods and digestibility results has been presented. It is recommended that preliminary periods in digestibility trials with cattle consist of nine days as a minimum.

A problem of long standing in animal nutrition is the determination of the true effect of added carbohydrate on forage digestibility. Diverse findings appear in the literature regarding this relationship. For instance, it has been shown that in some experiments added starch has produced little or no alteration in forage digestibility; whereas, in other cases, a marked decrease in digestibility has been observed.

The starch effect on forage digestibility can not be expressed as a simple quantitative relationship for, although it has been demonstrated that small amounts of starch (300 g. or less per day) in cattle had no effect on the digestibility of the forage (13, 16), other workers have shown that 5 or 6 lb. of starch per day may or may not produce a marked depression in digestibility (1, 3, 9, 10).

The variability of results for forage digestibility with starch has been reported to be influenced to a significant degree by the ash and nitrogen content of the forage (1, 2), both of which may have an effect on the flora of the intestinal tract. The problem is further complicated by the fact that starch also has a variable qualitative effect; sometimes it depresses the digestibility of all components of the forage (9, 10), and at other times lowers digestibility of the crude fiber component only (7, 11, 13, 15).

Since a review of the literature indicates that a daily intake of 1 lb. of starch would have little or no effect on forage digestibility, and the divergent forage digestibilities were usually found at the higher starch intakes, the plan of the experiment reported in this paper was to investigate the effect of starch on the digestibility of alfalfa at the 6-lb. level. This was accomplished by conducting a series of digestibility trials, with and without a starch supplement of 6 lb. a day, under varying conditions. An attempt was also made to discover which nutrients in alfalfa are most affected by increased intakes of readily digested carbohydrate.

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[1] Data presented are part of a thesis presented by the senior author in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of Georgetown University, Washington, D. C., 1957.

EXPERIMENTAL PROCEDURE

Three cows were fed, alternately, rations of alfalfa hay alone and alfalfa hay plus starch. The experiment consisted of four digestion trials. In the odd-numbered periods, the animals received an all-alfalfa ration and, in the even periods, alfalfa hay plus starch was fed. Daily supplements of steamed bonemeal (100 g.) and salt (50 g.) were provided. The alfalfa hay used was a U.S. No. 1 grade; the starch, a commercial cornstarch called Amazo. The amounts of alfalfa hay and starch fed, as well as the chemical composition of the rations, are reported in another paper (8).

Digestibilities were determined by the total collection method, using an automatic collection device. Collection periods were five days in Periods I and II, and six days in Periods III and IV. Preliminary periods varied for the four digestion trials in the following manner: 10, 20, five, and zero days.

Digestibilities reported are for alfalfa hay. An average of Morrison's (12) and Schneider's (14) digestibility values for starch was used to correct for indigestible starch residues.

When the digestibility trial on the all-alfalfa ration (Period I) had been completed, the amount of hay fed each cow was reduced 2 lb. per day, and starch was added to the hay ration in daily 1-lb. increments until 6 lb. of starch was fed. At this level, the starch content of the ration (dry basis) was 18% for the Holsteins and 22% for the Jersey. After a 20-day preliminary period, a five-day digestion trial on the starch-alfalfa ration (Period II) was carried out.

The digestion trial sequence of alfalfa hay alone, and alfalfa plus starch, was repeated in Periods III and IV, but with no time allowed between periods for animal adjustment to the ration change. While it is not good practice to run digestion trials without adequate preliminary periods, in this particular case the main portion of the ration (alfalfa hay) remained unchanged, and the only alteration made was the addition of starch, the immediate effect of which on alfalfa digestibility was the point at issue. The starch was not added abruptly, but at the following successive daily rates: 1, 3, 5, 6, 6, and 6 lb.

RESULTS AND DISCUSSION

The daily dry matter digestibilities found in Periods I through IV are reported in Tables 1–4, respectively. Although the daily dry matter digestibilities of the individual cows fluctuated, the average digestibilities of the cows for the periods were in good agreement. The means and standard errors of the alfalfa dry matter digestibilities for the four periods were, respectively: 60.2 ± 0.3, 59.8 ± 0.8, 61.0 ± 0.7, and 57.2 ± 1.4.

It was evident from the digestibility data in Tables 1 and 2 that the addition of 6 lb. starch to an all-alfalfa ration had no effect on the dry matter digestibility of the forage, when a 20-day preliminary period was allowed (Period II). It was also noted that the digestibility of the alfalfa for the first three days in Period IV averaged 61.6, S.E. $\pm$ 1.0, which indicated little change from Period III (61.0). However, in the last three days of Period IV, while the cows were

TABLE 1

Daily Dry Matter Digestibilities in Period I

Day	Cow 280	Cow 293	Cow 647	Average
1	57.7	59.5	62.4	59.9
2	60.1	60.1	64.7	61.6
3	65.4	63.8	59.8	63.0
4	56.4	58.0	53.3	55.9
5	61.0	59.8	60.8	60.5
Average	60.1	60.2	60.2	60.2

TABLE 2

Daily Dry Matter Digestibilities in Period II

Day	Starch fed	Cow 280	Cow 293	Cow 647	Average
	(lb.)				
1	6	64.0	62.1	56.2	60.8
2	6	62.0	59.5	60.4	60.6
3	6	58.5	56.1	59.6	58.1
4	6	60.8	60.5	59.1	60.1
5	6	55.9	55.4	66.8	59.4
Average		60.2	58.7	60.4	59.8

TABLE 3

Daily Dry Matter Digestibilities in Period III

Day	Cow 280	Cow 293	Cow 647	Average
1	57.0	60.9	63.3	60.4
2	60.9	59.8	58.4	59.7
3	62.0	60.6	62.1	61.6
4	66.4	62.6	57.7	62.2
5	66.2	59.8	60.0	62.0
6	54.3	63.3	63.0	60.2
Average	61.1	61.2	60.8	61.0

TABLE 4

Daily Dry Matter Digestibilities in Period IV

Day	Starch fed	Cow 280	Cow 293	Cow 647	Average
	(lb.)				
1	1	58.1	60.3	60.7	59.7
2	3	59.8	62.5	62.1	61.5
3	5	63.9	59.1	67.5	63.5
4	6	58.2	42.9	57.6	52.9
5	6	48.6	54.3	50.7	51.2
6	6	60.4	49.4	53.9	54.6
Average		58.2	54.8	58.8	57.2

receiving 6 lb. starch per day, the alfalfa digestibility averaged 52.9, with a standard error of $\pm$ 1.9, showing that the addition of starch did depress roughage digestibility. The decrease in alfalfa digestibility for the last three days of Period IV was highly significant ($P<1\%$).

The lowered dry matter digestibility of Period IV could not be due either to a protein or to a nitrogen deficiency, or to a lack of inorganic nutrients. The experimental animals received over 20 lb. alfalfa hay per day, supplemented by 100 g. of steamed bonemeal and 50 g. salt, which should provide more than an adequate supply of mineral nutrients. The nutritive ratio of the rations fed varied from 1:3.8 on the all-alfalfa ration to 1:6.6 on the alfalfa-starch ration, indicating an adequate protein allowance throughout the experiment.

Since the alfalfa fed in all the periods was from the same lot, and since the chemical analyses of the samples of hay from the various periods, except for ether extract, were in agreement, the only known cause for the divergent digestibilities of the starch supplement was the difference in the amount of time allowed the animals to adjust to the increased carbohydrate intake. When the preliminary period was 20 days, no digestibility effect for the added starch was realized. When the preperiod was zero-days, significantly lowered digestibles were found due to the added starch. Results indicate that the time period allowed for animal adjustment to ration changes may have an important bearing on conclusions drawn from feeding experiments with ruminants.

For instance, Huffman and Ducan (4), in their experiments on added starch, allowed no preliminary periods. The 15-day supplemental period, in which 6 lb. of starch was fed, was compared with the immediately preceding 15-day period without starch. These authors observed that the addition of 6 lb. starch did not increase the production of fat-corrected milk (FCM), even though the added starch raised the TDN intake. The subsequent replacement of the starch with the same amount of corn or wheat did increase the production of FCM appreciably, although the total TDN intake remained unchanged.

An explanation of Huffman and Ducan's results (4) could be as follows: Added starch did not increase FCM yield because starch, when added abruptly to a roughage ration, depressed roughage digestibility as in Period IV above. The increased FCM production observed by Huffman *et al.* (4), when corn grain in the succeeding period replaced starch, could be due to the fact that the animals had sufficient time, during the 15-day starch period, to adjust to starch, so that when the corn (70% starch) was fed, alfalfa digestibility was not depressed, as shown in Period II above. However, this explanation is inadequate, since Huffman and Duncan (5) have demonstrated in a subsequent experiment that corn grain, added to an all-alfalfa ration without any preliminary period, increased FCM production.

Digestibility results of Period II support the finding of Burroughs *et al.* (3), who fed start to cattle at 29, 44, and 55% levels and found that starch exerted little or no influence on the dry matter digestibility of good-quality alfalfa hay. Starch was fed from low to high percentages, so that the lower levels of starch intake could be considered as adjustment periods for higher starch intakes.

Burroughs' results indicated that a ten-day preliminary period was adequate for the starch levels fed, and also demonstrated the ability of the bovine digestive apparatus to utilize efficiently rations of high starch content, provided adequate adjustment time was permitted.

The importance of the adjustment period in digestibility studies has been provided by another experiment of Burroughs and associates (2), in which the purpose of the experiment was to discover what effect the water extract of alfalfa hay had on the dry matter digestibility of the basal ration. The average dry matter digestibilities for seven successive ten-day periods, without preliminary periods, were run in the following order: (1) basal ration, (2, 3, and 4) basal ration plus alfalfa water extract, and (5, 6, and 7) basal ration. The digestibility data showed that the second and fifth periods, which ordinarily would be preliminary periods due to ration changes, gave values in agreement not with the ration actually fed but with the ration fed in the previous ten-day period.

Further evidence regarding the significance of the adjustment period has been provided by Hungate *et al.* (6). These investigators had two of three sheep die of acute indigestion within 24 hr. after the ration had abruptly been changed from hay to grain. These workers reported that large quantities of starch or glucose lead to the production of lactic acid, which lowers the pH of the rumen liquor. The change in pH from slightly alkaline (normal) to acid (sometimes down to pH 4) results in a profound modification in rumen fermentation. These authors pointed out that most cases of ruminant acute indigestion were caused by ration changes, usually the substitution of a more readily digested feed for one less digestible.

It appears from the results of this experiment, and other available evidence, that the time factor required for adjustment to a ration change has a more significant impact on digestibility than previously has been supposed. It is known that the rumen bacteria appears to be influenced by the ration fed, both in regard to types and numbers. When drastic ration changes take place, considerable time may be required for the kinds of microorganisms to become stabilized to the new condition, so that a more suitable microflora may be assembled for the efficient digestion of the new ration. Therefore, it seems logical to conclude that digestibility data obtained in experiments without preliminary periods should be viewed as of questionable value.

In regard to which nutrient in alfalfa is most affected by addition of starch, the evidence of this paper is as follows:

TABLE 5

Alfalfa Component Digestibilities (Average of Three Cows)

Period	Dry matter	Protein	Ash	Ether extract	Crude fiber	NFE
I	60.2	69.4	23.1	14.0	42.3	73.6
II	59.8	66.2	27.0	13.4	44.0	72.5
III	61.0	69.1	24.3	30.0	48.0	72.3
IV	57.2[a]	63.2[a]	20.4	24.1	42.4	70.9[b]

[a] Significant at 5% level.
[b] Significant at 1% level.

In Table 5 can be found the average digestibility coefficients obtained in the four periods for the components of alfalfa. No significant differences among the component digestibilities were observed in the first three periods. In Period IV, highly significant differences were found for nitrogen-free extract, as well as significant differences for protein and dry matter digestibilities.

In Period II, with a 20-day preliminary period, no component of the alfalfa was significantly depressed by an addition of 6 lb. starch. A comparison of the data in Table 5 for Periods III and IV shows that with no preliminary period the digestibility averages of all forage components were depressed upon addition of starch. This finding is in agreement with Kuhn (10) and Kellner (9), both of whom have reported a depression in digestibility of all the ingredients of the basal ration upon addition of starch or other easily digested carbohydrate. However, in Period IV only protein, NFE, and dry matter were depressed significantly.

Mangold (11), Robinson (13), Swift *et al.* (15), and Kane *et al.* (7) have reported that the crude fiber component of the ration is the one most adversely affected by the addition of readily available carbohydrate. Although the crude fiber digestibilities of all three cows were lower in Period IV than in the previous period without starch, Cow 293 was the only animal to display a marked depression in crude fiber digestibility (25%). Cow 280 was 6% lower, and Cow 647, 2%. It should be noted that the component digestibility results in Period IV were taken from a six-day composite, in which the first three days, when 1, 3, and 5 lb. of starch were fed, showed normal dry matter digestibilities. This was in direct contrast to the depressed dry matter digestibilities found for Days 4, 5, and 6, when the full 6 lb. of starch were fed.

The conflicting evidence in the literature regarding the starch effect on forage digestibility may be due, to some extent, to differences in length of preliminary periods, but this is not the whole story. For, in the literature, experiments have been reported in which adequate preliminary periods have been observed and yet, serious component digestibility depressions have been found (7, 13, 15). The present authors (7) have found, in contrast to the starch-alfalfa digestibility relationship in Period II above that, after a nine-day preliminary period, corn grain added to grass silage lowered crude fiber digestibility significantly.

To explain these anomalous digestibility results, it is postulated that the starch in corn grain differs from commercial cornstarch in its effect on forage digestibility. It is known that treatment with dilute acid below gelatinization temperature (62–70° C.) causes random hydrolysis of corn starch. It appears likely, therefore, that the industrial process of exposing the starch in corn grain to steep liquor (0.15% SO_2) at a temperature of 50° C. for 40 hr. may result in some hydrolysis of the starch. It is also possible that interactions between concentrates and forages may affect apparent forage digestibilities. At the present time, it is not clear which of the two explanations is the valid one, and it may be that both are concerned.

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EFFECT OF HIGH-LEVEL ADMINISTRATION OF CHLORTETRACYCLINE AT BIRTH ON THE HEALTH AND GROWTH OF YOUNG DAIRY CALVES [1]

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SUMMARY

Therapeutic and nutritional aspects of the effect of chlortetracycline (CTC) on health, growth, and feed efficiency of 64 newborn Holstein and Jersey female and male calves were studied. Daily levels of either 0, 250, 500, or 1,000 mg. of the antibiotic administered for the first three days of life resulted in a reduction in enteritis and death in those calves receiving chlortetracycline, with the 250-mg. level being as effective as the two higher levels. The high-level three-day treatments of the antibiotic to dairy calves at birth did not result in any subsequent stimulation in growth up to 16 wk. of age.

Starting at four days of age, the feeding of daily nutritional levels of 50 mg. of chlortetracycline to one-half of the calves in each group produced a significant ($P < .05$) increase in growth of approximately 18% at 16 wk. of age when compared to the control subgroups, regardless of the first three-day high level treatments. An increase in feed consumption and in feed efficiency in the antibiotic-fed calves also was noted.

The mode of action of antibiotics in stimulating growth in young calves can not be explained entirely by a reduction in "disease level," and probably involves a metabolic or endocrine effect.

It is now recognized that certain antibiotics at nutritional levels of 20–80 mg. per day stimulate the growth of young calves (1, 3, 7, 8, 11, 13, 15, 17). Also, in most instances where gastrointestinal disease or diarrhea was indicated in calf experiments, it was reported that the antibiotic reduced the number of cases and the severity of the disease, or altered the intestinal flora, suggesting that this might be responsible for the growth stimulation (1, 7, 8, 9, 10).

It is not known definitely whether the observed reduction in the gastrointestinal disease or disease level is responsible for the growth stimulation in calves. On the basis of research from this station it has been suggested that the growth stimulation in young calves fed nutritional levels of antibiotics may be due to a metabolic or endocrine effect, rather than to a reduction in disease level or change in intestinal flora (5, 6, 14, 15). If a lowering of the disease level of calves is responsible for the growth stimulation of young calves, then prophylactic or therapeutic levels of the antibiotic used in the treatment or prevention of gastrointestinal disease also should result in a growth stimulation.

An opportunity to study this possibility was made available during the summer of 1954, when a high degree of enteritis appeared in the calves born at the L.S.U. Dairy Farm. New-born calves scoured and died during 1 to 2 wk. of life. In preliminary trials, using various therapeutic agents to combat this condition, chlortetracycline, at a level of 800 mg. daily for a three-day period, was found to be most effective.

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An experiment was designed to determine the minimal therapeutic level of antibiotic to use for prophylactic treatment of scours of new-born calves for three days. Also, a nutritional aspect was decided upon to determine whether the high-level feeding of the antibiotic for three days would have a carry-over effect and stimulate growth of calves, or whether supplemental nutritional levels (50 mg. daily) of the antibiotic after the high-level feeding would be required to stimulate growth significantly.

EXPERIMENTAL PROCEDURE

Sixty-four Holstein and Jersey new-born female and male calves were used in this trial. The calves, with restrictions as to breed and sex, were randomly assigned to one of eight treatments, consisting of one of four initial daily therapeutic levels of chlortetracycline, 0, 250, 500, and 1,000 mg., for the first three days of life and one of two subsequent daily nutritional levels, 0 and 50 mg., from the fourth day through the 16 wk. (Table 1). The antibiotic was adminis-

TABLE 1
General experimental design

Group and treatment (first 3 days)	No. of animals	Group and treatment after 4 days of age	No. of calves
I		A Control	11
No CTC[a]	18	B CTC 50 mg/day	7
II		A Control	8
CTC	16	B CTC	8
250 mg/day		50 mg/day	
III		A Control	9
CTC	16	B CTC	7
500 mg/day		50 mg/day	
IV		A Control	7
CTC	14	B CTC	7
1,000 mg/day		50 mg/day	

[a] Chlortetracycline.

tered orally in the form of oblets during the initial therapeutic period, and in the form of tablets during the subsequent nutritional period, until the starter consumption containing chlortetracycline approximately the 50-mg. intake level.

Feeding regime. All calves received colostrum at a level of 10% of the body weight for the first three days. Starting at four days of age, whole milk was fed up to 21 days of age, and then reconstituted skimmilk, one part to nine parts of water for 35 days (up to 8 wk. of age) at a level of 10% of the body weight. Good-quality alyce or alfalfa hay and a 23% plant protein calf starter [2] were fed to the calves beginning at four days of age. Subgroup A received the calf

[2] The starter consisted of the following percentages: cottonseed meal, 35.0; soybean oil meal, 5.5; yellow corn meal, 31.5; crimped oats, 15.0; wheat bran, 10.0; steamed bonemeal, 2.0; salt, 1.0, and Quadrex 10 (Type IV), 0.2; supplied by NOPCO Chemical Co., Harrison, N. J.

starter supplemented with ½% Aurofac 2A.[3] In addition, the calves in this group received a tablet containing 50 mg. chlortetracycline daily, until the calves were eating sufficient calf starter supplemented with Aurofac to approximate 50 mg. chlortetracycline. The starters were limited to 4 lb. per day for all calves, except the Holstein males, which received a maximum of 5 lb. per day. Hay was fed ad libitum. Body weights and measurements of height at withers were taken at birth and thereafter at weekly intervals through 16 wk. of age. Daily observations of each calf were made for any evidence of digestive disturbance, and all feed intakes were recorded. Data were subjected to statistical analyses, according to methods outlined by Snedecor (19). Arcsine transformation was used for the data on scours and mortality rate. Since death of calves resulted in unequal and disproportionate subclass numbers, particularly with reference to growth and feed efficiency data, the method of weighted squares of means was used.

RESULTS AND DISCUSSION

When high levels of chlortetracycline were administered to new-born calves for the first three days of life, a significant reduction ($P<0.05$) in mortality rate of the calves (Subgroups C) was obtained (Table 2). Calves in Group I (no CTC treatment) scoured mostly during the 1st wk. of life and prior to 2 wk. of age, while those on the antibiotic-treated groups (II to IV) scoured after 2 wk. of age. Thus, therapeutic high-level antibiotic treatment for new-born calves also appeared to be effective in warding off early incidence of scours,

TABLE 2

Effect of high-level feeding of antibiotics on scours and mortality rate of calves

Group and treatment (first 3 days)	Subgroups treatment after 4 days of age	No. of calves[a]	No. of calves scouring	Death to 8 wk. of age[b]	Per cent of	
					Scours	Death
I	C Control	9	6	4[d]	66.7	44.4
No CTC	A CTC 50 mg/day	7	1	1	14.3[e]	14.3[e]
II	C. Control	7	2[c]	1	28.6	14.3
CTC 250 mg/day	A CTC 50 mg/day	8	0	0	0.0[e]	0.0[e]
III	C Control	8	4[c]	2[d]	50.0	25.0
CTC 500 mg/day	A CTC 50 mg/day	8	1[c]	1	12.5[e]	12.5[e]
IV	C Control	6	3[c]	1	50.0	16.7
CTC 1,000 mg/day	A CTC 50 mg/day	7	0	0	0.0[e]	0.0[e]

[a] Death due to unknown causes was taken out of number of calves.
[b] Death due to enteritis (scours).
[c] Scours after 2 wk. of age.
[d] One animal of group also contracted pneumonia and died.
[e] Highly significant differences ($P<0.01$) on the incidence of scours and mortality were found between all groups receiving nutritional levels (50 mg.) of the antibiotic and the control group.

[3] Supplied by the American Cyanamid Co., Pearl River, N. Y., contains 3.6 g. Aureomycin per pound.

although no statistically significant differences were obtained between the groups. The 250-mg. level of chlortetracycline was as effective as higher levels of the antibiotic ($P<0.05$) in preventing death in new-born calves suffering from enteritis (scours) up to 8 wk. of age. Bortee *et al.* (2) and Roy *et al.* (12) also have reported that high levels of chlortetracycline at birth reduced the incidence of scours and death.

The daily feeding of a nutritional level of chlortetracycline (50 mg.) at four days of age markedly reduced the number of cases and incidence of scours as well as deaths. These differences were found to be highly significant at the 1% level of probability (Table 2). A lowering of the mortality rate of the calves in Subgroup A of Control Group I was obtained by the daily feeding of the nutritional level of the antibiotic; however, some intestinal disturbance occasionally was in evidence among the calves. Many of the calves in Groups I to IV which did not receive the nutritional level of chlortetracycline after the therapeutic high-level treatment (Subgroups C) showed evidence of scours and a few animals died between 2 and 8 wk. of age.

The effect of the therapeutic high-level treatment for the first three days of life on the weight gains of the calves (Subgroups C) at 16 wk. of age is shown in Table 3. Only one calf in each of the antibiotic-administered groups outgained by a definite margin the calf in the control group which made the highest gains, as can be observed under the range of values. However, the average gains in weight for each group were similar and no significant differences were found.

TABLE 3

Effect of high-level treatment of antibiotic the first three days of life on gain in weight of dairy calves at 16 wk. of age

Group	Level of chlortetracycline	No. of animals	Gain in weight (0–16 wk.)	
			Range	Average
	(mg/day)		*(lb.)*	
I	0	4	76–101	89
II	250	7	73–110	90
III	200	5	66–122	99
IV	1,000	6	60–137	90

TABLE 4

Effect of a daily nutritional level of chlortetracycline supplement at four days of age on the average growth rate of Holstein calves

Treatment and group	Sex	Average gain in weight (lb.) Weeks of age			
		0–3	0–8[a]	0–12[a]	0–16[a]
Control	M	0.5	33.5	70.3	109.3
	F	4.8	38.6	67.8	100.4
	Average	2.6	36.0	69.0	105.0
Chlortetracycline *(50 mg/day)*	M	4.5	52.5	88.1	129.0
	F	4.8	44.3	87.7	129.6
	Average	4.7	48.0	88.0	129.0

[a] Significant difference between groups at $P<0.05$.

Thus, it appears that the high-level therapeutic treatment of the antibiotic for the first three days of life had no carry-over effect in stimulating the growth of calves up to 16 wk. of age.

The effect on the growth of the calves (Subgroups A) of a daily nutritional level of chlortetracycline supplementation, beginning at four days of age, is shown in Table 4 for the Holsteins and in Table 5 for the Jerseys. A significant advantage ($P<.05$) in weight gains in favor of the antibiotic-fed Holstein calves by 22% was found at 16 wk. of age; a 14% increase in weight gains was found in favor of the antibiotic-fed Jersey calves at 16 wk. of age. This latter increase statistically approached the 5% level of probability.

TABLE 5

Effect of a daily nutritional level of chlortetracycline supplement at four days of age on the average growth rate of Jersey calves

Treatment and group	Sex	Average gain in weight (lb.) Weeks of age			
		0–3	0–8[a]	0–12[a]	0–16[a]
Control	M	2.7	27.5	53.7	86.5
	F	3.4	25.6	58.9	83.9
	Average	3.0	26.0	56.0	85.0
Chlortetracycline *(50 mg/day)*	M	2.8	34.1	66.1	105.1
	F	3.2	30.8	59.6	88.7
	Average	3.0	32.0	63.0	97.0

[a] Differences in weight gains between groups approached significance at $P<0.05$.

At 16 wk. of age, the effect of the daily aureomycin supplementation on the height at withers of the calves showed no significant differences, the average values being 76 cm. for the control animals and 76.5 cm. for the antibiotic-fed animals.

The average feed consumption and feed efficiency of the antibiotic-fed and control calves is shown in Table 6. The antibiotic-fed animals consumed less whole milk but more skimmilk, grain, and hay than the control animals. Hay consumption was approximately the same for both groups; however, there were large individual differences in conssumption of hay within groups. When feed efficiency is expressed as pound of calf starter per pound of gain, the supple-

TABLE 6

Average feed consumption and efficiency of feed utilization of dairy calves at 16 wk. of age

Treatment and group	Breed	Average consumption per group (lb.)				Feed efficiency
		Whole milk	Skim-milk	Grain	Hay	Pound of grain per pound gain
Control	Holstein	148	309	270	77	2.60
	Jersey	115	221	227	54	2.67
	Average	131	265	248	65	2.63
Chlortetracycline *(50 mg/day)*	Holstein	139	322	294	77	2.27
	Jersey	108	232	242	65	2.57
	Average	123	277	268	71	2.42

mented group of calves showed a better feed efficiency, 2.42 lb., as compared to 2.64 lb. for the control group.

The administration of chlortetracycline to new-born calves resulted in a two-fold effect: *(a)* as a successful therapeutic agent (high-level dose) during the early life of the calf when it is under greatest stress, due to possible gastrointestinal disturbances and *(b)* as a growth stimulant when fed at daily nutritional levels.

The mode of action of antibiotics in stimulating growth in young calves is difficult to explain. Although high-level administration of chlortetracycline reduced the disease level drastically, some gastrointestinal disturbance still occurred after three days of age unless daily nutritional levels (50 mg.) were fed (Table 2). This would seem to indicate that the high-level dose of the antibiotic was excreted rapidly and reinfection could be possible after administration of the dose stopped. The daily nutritional level of 50 mg. of the antibiotic appeared to be sufficient to control any further gastrointestinal infection which might also be responsible for the growth stimulation obtained.

Recent work (17) at this station has shown that daily administrations of a high level (250 mg.) of chlortetracycline to new-born calves for 28 days was equally as effective in stimulating growth as was a three-day administration, followed by a daily nutritional level of 50 mg.

Thus, it is possible that a daily nutritional level of the antibiotic could control the latent disease level in normal calves. The administration of high levels of chlortetracycline to combat intestinal infections in the very young calf did not subsequently increase the growth rate of calves in this study, even though the disease level was drastically reduced. As suggested in earlier reports (5, 6, 14, 15), the mechanism of action of antibiotics in young calves in stimulating growth may also involve a metabolic or endocrine effect and further research is needed to substantiate this theory.

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TECHNICAL NOTES

INFLUENCE OF ADDED 1-MONOGLYCERIDES ON THE SURFACE TENSION OF MILK

In a study of the influence of monoglycerides on foaming in raw milk, surface-tension determinations indicated that some of these compounds decreased the surface tension of the milk. Herrington (3) suggested that the mono- and diglycerides produced during the lipolysis of milk may play an important role in reducing its surface tension. Certain monoglycerides have been shown to be surface active (2). Since monoglycerides have been found in lipolyzed milk (4), they should have an influence on the surface tension.

Preliminary trials with eight composite samples of milk taken from four Holsteins showed that when two drops (about 80 mg.) of 1-monoolein, 1-monolaurin, 1-monopalmitin, and 1-monostearin were stirred separately into 100-ml. milk samples, the average decrease in surface tension was 6.3, 5.2, 0.9, and 0.4 dynes per cm., respectively. The purities of these compounds were 92, 84, 94, and 70%. The surface tension was measured with a Cenco du Nuoy tensiometer calibrated to read in absolute surface tension.

To explore these decreases more thoroughly, 1-monolaurin and 1-monoolein were dissolved in ethanol and 0.2, 1.0, and 2.0 m*M* per 100 g. of fat were added to pasteurized whole milk. The monoglycerides were dissolved in ethanol so that they would be more readily dispersed in the milk. The additions were arranged so that 1 ml. of alcohol was the maximum amount added to 100 ml. of milk. Pasteurized milk was selected to avoid any lipolysis which could lower the surface tension of the samples. The samples were mixed, tempered to 20° C., and the surface tension measured. The results in Table 1 show that even very small quantities of monolaurin and monoolein reduced the surface tension of the samples. The results support the belief that at least part of the decrease in surface tension of lipolyzed milk is due to monoglycerides. The authors have detected about 2.0 m*M* of monoglyceride per 100 g. of fat in extremely rancid milk; whereas, fresh raw milk has been found to contain about 0.02 to 0.04 m*M* per 100 g. of fat. Thus, milk in various stages of lipolysis can contain small but effective amounts of surface active monoglycerides which do influence surface tension and foaming (1); and could influence whipping, the fat globule surface, and other surface and interfacial phenomena.

TABLE 1
Influence of added 1-monoglycerides on the surface tension of pasteurized milk

Samples[a]	Surface tension[b] Range	Average
Normal milk	44.6–46.4	45.6
Normal milk + 1 ml. ethyl alcohol	44.8–46.0	45.4
Monoolein added[c]		
0.2 m*M*/100 g. fat	43.1–44.5	43.7
1.0 m*M*/100 g. fat	38.9–41.6	40.3
2.0 m*M*/100 g. fat	37.9–40.1	38.5
Monolaurin added[c]		
0.2 m*M*/100 g. fat	43.3–45.7	44.3
1.0 m*M*/100 g. fat	40.9–42.5	41.9
2.0 m*M*/100 g. fat	37.3–40.4	39.2

[a] Five replicates on each.
[b] Readings in dynes/cm at 20° C.
[c] Monoglycerides dissolved in ethyl alcohol and 1 ml. added per 100 ml. of milk.

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INHIBITION OF LIPASE AND LIPOLYSIS IN MILK WITH N-ETHYLMALEIMIDE [1]

Milk lipases have been shown to be sensitive to heat, light, hydrogen peroxide, and copper (4, 5, 10, 11). Their storage stability has been markedly increased by reducing agents and glutathione (5). It has been shown (1, 9) that the activity of a large number of enzymes demands unobstructed sulfhydryl groups on the proteins. Frankel and Tarassuk postulated that these sulfhydryl groups are essential for milk lipase activity (5). Experiences in this laboratory and observations of others (8) indicate that *p*-chloromercuribenzoate reacts slowly, if at all; N-ethylmaleimide (NEM)[2] has been shown (6, 7) to react quickly and specifically with sulfhydryl groups under mild conditions. NEM was used as an inhibitor in the present investigation of whether the sulfhydryl groups are actually involved in lipolysis and lipase activity in milk. Freshly drawn, uncooled milk, mixed or from single cows, was obtained in a stainless-steel can and processed immediately after milking. The NEM was added directly to whole milk and the milk was incubated at 37° C. for a certain period before being subjected to activation treatments. For assay of lipase activity the NEM was added to skimmilk. The extent of lipolysis was measured by determination of free fatty acids (FFA) by an extraction-titration method (2). The lipase activity toward milk fat was assayed by the method of Frankel and Tarassuk (3). The method used to induce lipolysis in milk has been described previously (11).

N-ethylmaleimide inhibited both the lipase activity and the level of induced lipolysis (Table 1). Lipase activity was inhibited more than was lipolysis, perhaps because of the protective effect of the membrane surrounding the globular fat (5). The data of Table 2 show that incubation for 3 hr. at 37° C. with NEM (0.02 *M*) completely inhibited lipolysis induced by shaking, temperature-fluctuation, and homogenization. Such inhibition did not occur at 0–5° C. incubation with NEM. The degree of inhibition was quite similar among the three treatments at each concentration of NEM tested. Inhibition was much less effective when NEM was added after activation by shaking, perhaps because of the greater resistance of the lipase-substrate complex to

[1] This study was supported in part by funds from the California Dairy Industry Advisory Board.

[2] Obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio.

TABLE 2
Effect of NEM on induced lipolysis

N-ethylmaleimide	Increased FFA titration value	Inhibition
(M)	*(ml. of 0.025 N KOH per 10 ml. of milk)*	*(%)*
Shaking[a]		
0.0	2.10	0
0.4×10^{-2}	1.23	41
0.8×10^{-2}	1.05	50
1.2×10^{-2}	0.72	66
1.6×10^{-2}	0.24	89
2.0×10^{-2}	0.00	100
Temperature		
0.0	0.54	0
0.4×10^{-2}	0.24	56
0.8×10^{-2}	0.21	61
1.2×10^{-2}	0.06	89
1.6×10^{-2}	0.00	100
2.0×10^{-2}	0.00	100
Homogenization		
0.0	1.20	0
0.4×10^{-2}	0.75	42
0.8×10^{-2}	0.27	79
1.2×10^{-2}	0.21	84
1.6×10^{-2}	0.03	96
2.0×10^{-2}	0.00	100

Fresh whole milk was kept for 3 hr. at 37° C. with NEM, and subjected to various activation treatments, and after activation the milk was kept at 0–5° C. for 24 hr.

[a] Shaken for 60 min.

TABLE 1
Effect of NEM on lipase activity and lipolysis of the same milk

N-ethylmaleimide	Lipase activity		Lipolysis	
	Activity units	Inhibition	Increased FFA titration value	Inhibition
(M)	*(per 100 ml. milk)*	*(%)*	*(ml. of 0.025 N KOH per 10 ml. of milk)*	*(%)*
0	2.27	0	1.56	0
1.0×10^{-2}	1.24	45	1.14	27
2.0×10^{-2}	0.56	75	0.80	49

Fresh whole milk and skimmilk from the same origin were kept at 37° C. for 1 hr. with NEM, then whole milk was activated by shaking for 1 hr. at 37° C. Skimmilk was assayed for lipase activity.

TABLE 3
Effect of NEM added before and after activation

N-ethylmaleimide	Before[a] Increased FFA titration value	Before[a] Inhibition	After[b] Increased FFA titration value	After[b] Inhibition
(M)	*(ml. of 0.025 N KOH per 10 ml. of milk)*	*(%)*	*(ml. of 0.025 N KOH per 10 ml. of milk)*	*(%)*
0	0.75	0	3.24	0
2.0×10^{-2}	0.00	100	2.43	25

[a] Fresh whole milk was kept for 3 hr. at 37° C. with NEM and shaken for 1 hr. at 37° C., then immediately cooled and kept at 0–5° C. for 24 hr.
[b] Fresh whole milk was shaken immediately after milking for 1 hr., then NEM was added and kept for 3 hr. at 37° C. before cooling, and kept at 0–5° C. for 24 hr.

the inhibition. Adding glutathione (0.01 *M*) to milk with NEM (0.01 *M*) reduced inhibition by 76%.

Inhibition of spontaneous lipolysis was studied with milk that for over 3 mo. had a history of spontaneous rancidity, recognized by both organoleptic and chemical means. Complete inhibition was obtained after 1 hr. of incubation at 37° C. with NEM (0.02 *M*). This suggests that spontaneous lipolysis is more susceptible to NEM inhibition than is induced lipolysis.

TABLE 4
Effect of glutathione on the inhibition of lipolysis by NEM

N-ethylmaleimide	Glutathione	Increased FFA titration value	Inhibition
(M)	*(M)*	*(ml. of 0.025 N KOH per 10 ml. of milk)*	*(%)*
0	0	0.51	0
1.0×10^{-2}	0	0.06	88
0	1.0×10^{-2}	0.57	0
1.0×10^{-2}	1.0×10^{-2}	0.45	12

Fresh whole milk was kept at 37° C. for 30 min. with NEM in 0.2 *M* phosphate buffer, pH 6.6, then GSH was added and incubated for 2.5 hr. Prior to activation of shaking at 37° C. for 1 hr., pH was readjusted to 6.6 with 1 *N* NaOH electrometrically.

TABLE 5
Effect on spontaneous lipolysis

Incubation period before cooling	Increased FFA titration value: Without N-ethylmaleimide	Increased FFA titration value: With N-ethylmaleimide	Inhibition
(hr.)	*(ml. of 0.025 N KOH per 10 ml. of milk)*		*(%)*
0	0.42	0.03	93
1	0.33	0.00	100
2	0.27	0.00	100

Fresh whole milk from an individual cow was kept at 37° C. with or without NEM (0.02 *M*), cooled, and kept at 0–5° C. for 24 hr.

It is concluded that the sulfhydryl groups are essential both for induced and for spontaneous lipolysis, that they are likely involved in the formation of the specific lipase-substrate complex, and that the active sites of the lipases are either at or near the sulfhydryl groups.

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TESTS TO MEASURE SYNERESIS AND FIRMNESS OF COTTAGE CHEESE COAGULUM, AND THEIR APPLICATION IN THE CURD-MAKING PROCESS[1]

Whitaker (6) and others have pointed out that certain lots of reconstituted nonfat dry milk (NDM) are difficult to use in making cottage cheese. The manufacture of miniature batches of cottage cheese can be used to evaluate the cottage cheese–making characteristics of reconstituted NDM. However, the time and special techniques required by such a method and the difficulty of interpreting the results make it desirable to search for simpler, less laborious methods. Since the curd from desirable reconstituted NDM expels whey rapidly and is firm at cutting, tests which measure these characteristics might be used to evaluate NDM for cottage cheese.

The purpose of this report is to describe a test, its application and relative accuracy for measuring the moisture-expelling characteristics of cottage cheese curd.

In this discussion, the term coagulum refers to skimmilk gel from coagulation to the start of heating; from that point, the gel is called curd. Curd strength refers to the strength of the coagulum at cutting.

METHODS

Drainage test. The equipment used in the drainage test is shown in Figure 1.

FIG. 1. Equiment for the drainage test: Left—beaker of coagulum with cutter and spatula in place for use; center—cutting device; right—whey-separation pan and paddle being used to manipulate the coagulum.

In performing the drainage test, 360 ml. of skimmilk containing the coagulant (rennet, lactic culture, or both) were placed in a 400-ml. beaker and held at 90° F. until coagulation occurred.

An inexpensive cutter was made to produce a uniform cutting pattern in all tests. A separate cutter was used in each test when several tests were made simultaneously. Each cutting device was made by cutting the bottom out of a new 202 × 314 tin can; the opposite end of the resulting cylinder was shaped to give it a cutting edge. From an identical can two pieces of metal were cut, flattened, and shaped to fit inside the cylinder and to divide its interior into quarters.

A whey-separation pan was made of a shallow, aluminum tray approximately 12 in. long, 5 in. wide, and 1¾ in. deep. One 12-in. side was cut at each end and flattened to open the tray and facilitate the separation of curd from whey. A 3-in.-wide, flat square-ended paddle was used to manipulate and separate the curd from the whey on the tray. In separating the curd from the whey, the tray was tilted so that the open side was the high side.

At the time chosen for cutting, the cutter was forced down vertically through the coagulum as close to the center of the beaker as possible and left there to prevent matting of the freshly-cut surfaces. A thin-bladed spatula was used to cut it away from the edge of the beaker. This cutting operation produced four pieces of coagulum of equal size surrounded by a cylinder of coagulum approximately ½ in. thick. The beaker of coagulum with the cutter in place was left in the water bath at 90° F. for 1 hr., a holding time selected after preliminary experiments.

After the 1-hr. holding period, the cutter was removed, the beaker was tipped, and the coagulum and whey were carefully slipped from the beaker to the sloping whey-separation pan with as little breakage as possible. Each large piece of coagulum was gently moved with the paddle to the upper, open side of the tray, drained momentarily, then pushed out of the tray over the open side and discarded. All the coagulum was removed within a period of 60 sec. after the contents of the beaker had been placed on the tray. This time limit was observed to minimize the amount of whey exuded by the coagulum during its mechanical separation from free whey on the pan. This was especially important if there happened to be some breakage of the coagulum during tilting, and whey separation manipulations.

The whey, which had been expelled in the 1-hr. drainage, was collected in the lower side of the tray; it was then poured into a graduated cylinder through a fine-meshed screen which removed small pieces of coagulum. The whey expelled from 360 ml. of coagulum was measured in milliliters and recorded as the drainage test.

Variability of drainage test. A rennet coagu-

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lum was used for one series of tests to measure uniformity under conditions of rapid whey expulsion. The coagulum was formed from 360 ml. raw skimmilk at 90° F. by adding 2 ml. of a 5:100 solution of rennet in water; it was cut 1 hr. after adding the rennet, and the drainage test was completed in the manner which has been described. The average drainage obtained from eight replicate tests of identical samples was 87.3 ml., with a standard deviation of the observations of ± 2.55 ml. A second series of tests for uniformity was made with normal cottage cheese coagulum, obtained from pasteurized skimmilk with the customary amounts of rennet and lactic culture. The average drainage test for six tests of identical samples was 51.8 ml., with a standard deviation of the observations of ± 3.43 ml.

Curd strength. The curd strength of the coagulum in 250-ml. beakers was measured with the Cherry-Burrell curd tension apparatus (1). The coagulum was formed in the same manner as that for the drainage test.

Acid-coagulum (A-C test). The A-C test (2) was performed in a 250-ml. beaker, which was held in the same water bath containing the beakers of skimmilk for the drainage test and the curd strength measurement.

pH determinations. A Beckman Model G pH meter was used with an external glass electrode and an external sleeve-type reference electrode.

RESULTS AND DISCUSSION

Certain factors which influence syneresis and firmness of cottage cheese curd were varied to measure their effects on curd strength and whey drainage. These factors were pH of cutting, total solids of the skimmilk, the level of rennet used, and the variability of the skimmilk itself.

Acidity at cutting. The effect of the acidity of the coagulum at the time of cutting on the drainage test and curd strength were observed by using two concentrations of reconstituted nonfat dry milk; one contained 8.8, the other 11.0%, solids. Each lot of skimmilk was coagulated with rennet and lactic acid culture as in the making of cottage cheese. A series of tests was performed with each lot, so that each test was made at a different level of pH in the coagulum. Curd strength tests were made on the coagulum formed from the reconstituted NDM containing 11% solids. The results, which are typical of other comparable observations, are shown in Table 1.

The amount of whey expelled in the drainage test decreased as the pH was decreased at the time of cutting. It was not unexpected, because it is common knowledge that excessive acidity at cutting delays the firming of curd during the heating process in making cottage cheese. The data of Table 1 show that the curd strength increased as the pH at cutting decreased. This observation confirms the work of Heineman (3).

TABLE 1

Effect of pH at cutting on drainage test and curd strength of cottage cheese coagulum

Coagulum with 8.8% T.S.[a]		Coagulum with 11.0% T.S.[a]		
Acidity at cutting	Drainage test	Acidity at cutting	Drainage test	Curd strength
(pH)	*(ml.)*	*(pH)*	*(ml.)*	*(g.)*
4.92	77	4.83	37	45
4.87	71	4.78	38	58
4.74	51	4.71	28	62
4.74	50	4.66	29	92
4.73	54	4.59	26	112
4.68	48			
4.63	40			

[a] Coagulum formed at 90° F. by adding 5% starter culture, followed 1.5 hr. later by rennet added at the rate of 1 ml. per 1,000 lb. of the reconstituted skimmilk.

Total solids effect. Drainage and curd strength measurements were made on a series of samples of NDM after reconstituting it to different levels of total solids. Tests were made on coagulum produced by lactic culture and rennet as in making cottage cheese by the short-set method. The A-C end-point determined the proper stage of coagulation for making these tests.

Increasing the total solids of reconstituted NDM, as shown in Table 2, increased curd strength at the A-C end-point, but decreased the whey-expelling powers of the coagulum. This does not seem to agree with the observations of Mykleby and Zakariasen (4), who reported that high solids curd firmed more rapidly and with less heat. Yet these diverse observations may not necessarily be contradictory, since cottage cheese curd made from skimmilk with high total solids content does not have to expel as much whey to reach the desired total solids and firmness as does curd made from skimmilk with low total solids.

Rennet effect. Drainage and curd strength tests were made from reconstituted NDM co-

TABLE 2

Effect of total solids on curd strength and the drainage test at the A-C end-point in acid-rennet coagulum[a]

Total solids in skimmilk	Acidity at A-C end-point	Drainage test	Curd strength
(%)	*(pH)*	*(ml.)*	*(g.)*
5.8	4.83	96	20
7.7	4.78	65	35
9.6	4.75	47	54
11.5	4.70	29	65
15.3	4.61	11	80

[a] Coagulum formed at 90° F. from reconstituted NDM by adding 5% starter culture, followed 1.5 hr. later by rennet added at the rate of 1 ml. per 1,000 lb. of skimmilk.

agulated as in making cottage cheese by the short-set method, but with amounts of rennet varying from 0 to 4 ml. per 1,000 lb. of milk. All tests were made at the A-C end-point. The data in Table 3 show that increasing amounts of rennet increased curd strength and the amounts of whey released in the drainage test. But this relationship between syneresis and amount of rennet did not obtain when similar coagulum was heated in the cottage cheese curd-making operation. Three vat-lots of reconstituted NDM with 10.9% solids were made into cottage cheese, with rennet added at the rates of either 0.5, 1.0, or 2.0 ml. per 1,000 lb. of skimmilk. The vats were treated alike, yet the curd they produced, listed in order of increasing amounts of rennet, contained 20.1, 19.8, and 17.9% total solids. Thurston and Gould (5) reported a similar retardation of firming effect when they used excessive amounts of rennet during the manufacture of cottage cheese from skimmilk.

Effect of milk. Four lots of skimmilk were obtained at different times from the mixed milk of approximately 25 herds. All lots were pasteurized, condensed at 80° F. without preheating, and standardized with distilled water to 8.8% solids-not-fat. These four lots of skimmilk were coagulated for cottage cheese by the action of 5% starter at 90° F. and 1 ml. of rennet per 10,000 lb. of milk. Tests for drainage and curd strength were coagulated in the same manner. The cottage cheese coagulum was tested and cut at the A-C end-point and the same procedures of cutting, heating, draining, and washing were used in making each vat of curd into cheese. The observations on these lots are shown in Table 4.

Rates of syneresis of the coagulum from the four lots of skimmilk varied widely as measured by the drainage test; the amount of whey accumulated ranged from 11 to 71 ml. Curd strength tests of the coagulum from the several lots of skimmilk also showed wide variations, ranging from 5 to 90 g. Maximum and minimum values in the drainage test were associated with maximum and minimum values, respectively, of curd strength measurements. Such variations of curd strength in different lots of milk often have been observed when the cheese has been made with 1 ml. of rennet per 1,000 lb. of skimmilk.

The variations in curd strength and drainage tests shown for different lots of milk in Table 4 resemble the trends and the magnitude of variations in these same tests obtained by using different amounts of rennet, as shown in Table 3.

It is highly significant for the objectives of this study that these measurements of curd strength and whey drainage showed no apparent relationship to the curd-making properties of the four lots of skimmilk under consideration. The skimmilk of Lot B, which had the lowest curd strength and lowest value for the drainage test, was made into cheese without difficulty and without unusual or excessive curd losses. The total solids content of the curd from Lot B was similar to that produced by Lot C, which had high curd strength and a high drainage test at the time of cutting.

TABLE 3

Effect of rennet additions on curd strength and the drainage test measurements on acid-rennet coagulum[a] cut at the A-C end-point

Concentration of rennet	pH at A-C end-point	Drainage test	Curd strength
(ml/1,000 lb.)		*(ml.)*	*(g.)*
0	4.69	?[b]	5
0.5	4.69	11	22
1.0	4.70	22	35
2.0	4.69	30	110
4.0	4.70	44	145

[a] The coagulum was formed by adding 5% starter culture and rennet 1.5 hr. later to NDM reconstituted at the rate of 11.5 lb. of NDM to 88.5 lb. of water.

[b] The soft coagulum broke so easily that separation of the whey for measurement was impossible.

CONCLUSIONS

Under the conditions of these experiments, the drainage test and curd strength of cottage

TABLE 4

Effect of different lots of pasteurized skimmilk on curd strength and drainage test at the A-C end-point of an acid-rennet coagulum and on the total solids of the finished cottage cheese curd

Skimmilk lot	pH at A-C end-point	Drainage test	Curd strength	Total solids of skimmilk	Total solids of curd
		(ml.)	*(g.)*	*(%)*	*(%)*
	4.71	69	90	8.8	23.1
A	4.70	62	70	8.8	23.9
	4.75	68	80	8.8	24.6
B	4.71	13	5	8.8	21.1
	4.72	11	9	8.8	21.4
C	4.71	71	68	8.8	20.7
D	4.76	64	62	8.8	25.3

cheese coagulum at the time of cutting tended to vary directly—but not uniformly—with variations in acidity of coagulum at the time of cutting, and with amounts of rennet used to form the coagulum. Increases in total solids in skimmilk increased curd strength but decreased drainage test values.

Variations in neither test seemed to be related to curd-making properties of the skimmilk used in these trials.

These observations indicate that these tests used under the conditions of these trials can not hopefully be considered to be of value for predicting the whey-expelling properties of cottage cheese curd made from reconstituted nonfat dry milk.

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STUDIES ON MILK POWDERS. II. A METHOD FOR THE ESTIMATION OF THE WETTABILITY OF MILK POWDERS[1]

The wettability of milk powder is an important consideration in the development of a readily dispersible whole milk powder. In the course of an investigation of the factors that may affect the wettability of whole milk powder, it was necessary to select or devise an objective test of wettability which would be reliable and convenient and at the same time capable of detecting small differences in wettability.

Several methods for the estimation of wettability, including the ones employed by Ashworth and Gunthardt (1), Kleinert (3), and Julien and Baker (2), were tried. Finally, a modification of the one proposed by Kleinert was found to be more suitable for our purposes, and this modified method is described in the present paper. The wettabilities, dispersibilities, and solubility indices of a number of commercial milk powders are reported, as well as results which demonstrate the effects of particle size on wettability and dispersibility.

METHODS

Wettability. A modification of the method of Kleinert (3) for the measurement of static dispersion or wettability was employed. A diagram of the apparatus is shown (Figure 1). An absorption tube (50 ml.) containing distilled water (30 ml.) (25° C.) was placed in the Evelyn Colorimeter (A). The instrument was adjusted to read 100% transmission. The glass tube (B) (1 in. by 26 in.) carried a powder funnel (C) at the top and a wire gauze (3-mm. opening) fitted in a ball-and-socket joint (Joint No. 35/20) (D) at a distance of 2.5 in. from the bottom. This tube was adjusted in a perpendicular position over the absorption tube. The sample holder (E) was filled level-full (1.8 ml.; 0.5–1.0 g.) with the milk powder and was adjusted over the glass tube (B). On lifting the lever (F), the sample holder (E) turned in the direction of the arrow and the contents fell through the tube and wire gauze and upon the surface of the water in the absorption tube. Light transmission measurements were made after 10, 15, 20, 30, 45, and 60 sec. The procedure was repeated four times for each milk powder and the average transmission readings for each time interval were plotted as ordinates against time as abscissae. A line was drawn horizontally through the 100% transmission point to meet a line drawn vertically through the 60-sec. point so as to form a rectangle bounded

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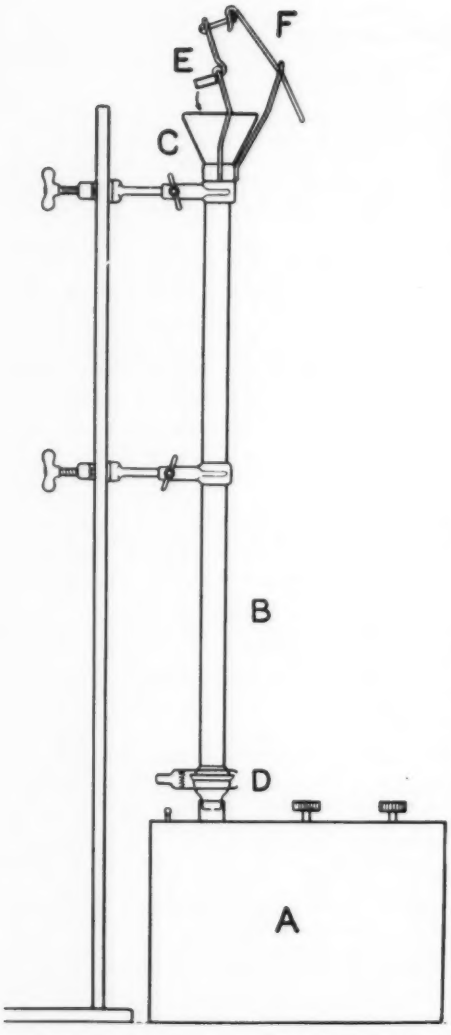

FIG. 1. Apparatus for the measurement of wettability.

by these two lines and the axes (axis of ordinates 0-100; axis of abscissae 5-60). The area between the curves and the foregoing two lines was measured and expressed as a percentage of the area of the whole rectangle. This percentage was adopted as the measure of wettability. The milk powders were maintained at 25° C. for at least 48 hr. before their wettabilities were determined.

Dispersibility. Water (50 ml.) (25° C.) was placed in a beaker (400 ml.) and a mechanical stirrer was adjusted so that the blades were close to the bottom but did not touch the bottom of the beaker. The speed of the stirrer was set at 250 r.p.m. by means of a rheostat. The milk powder (5 g.) was placed on the surface of the water and the stirrer was allowed to run for exactly 10 sec. The dispersed milk was quickly filtered through a fritted glass funnel (coarse) with the aid of suction. Portions (5-ml.) of the filtrate were transferred to weighed aluminum dishes which were then heated in an air-oven at 98–100° C. for 3 hr., cooled in a desiccator, and weighed. The ratio of the weight of the residue to the weight of sample represented by the 5-ml. portions, expressed as per cent, was taken as a measure of the dispersibility of the sample. The milk powders were maintained at 25° C. for at least 48 hr. before their dispersibilities were determined.

Solubility. The solubility of the milk powders was measured by the ADMI method.

RESULTS AND DISCUSSION

Table 1 shows the results of duplicate determinations of wettability of the various milk powders. The dispersibility values represent the average of three determinations, and the solubility indices represent single measurements. It will be noted that the duplicate values for wettability check fairly closely and are of the same order of magnitude, with the exception of Sample C, as the results reported for the dispersibility of the various milk powders.

Effect of particle size. Four commercial milk powders were fractionated as to particle size by means of the following sieves: U. S. Bureau of Standards No. 10 (2.0 mm.), No. 14 (1.41 mm.), No. 18 (1.00 mm.), No. 35 (0.50 mm.), No. 60 (0.25 mm.), No. 100 (0.149 mm.), and No. 200 (0.074 mm.). Table 2 shows the distribution of particles of the various sizes in the three milk products and Table 3 shows the effect of particle size on the

TABLE 1
Properties of commercial milk powders

Milk powder	Wettability	Dispersibility	Solubility index
	(%)	(%)	
A. Instant skim-milk powder	89; 90	82	0.08
B. Instant skim-milk powder	87; 88	77	0.10
C. Instant skim-milk powder	50; 51	74	0.08
D. Instant skim-milk powder	70; 72	72	0.05
E. Powdered skim-milk	49; 50	54	0.10
F. Powdered whole milk	13; 13	16	0.2
G. Agglomerated whole milk powder	2.9; 2.9	4.5	0.2

TABLE 2

Fractionation of milk powders as to particle size

Milk powder	Greater than 2.0 mm.	2.0–1.41 mm.	1.41–1.00 mm.	1.00–0.50 mm.	0.50–0.25 mm.	0.25–0.149 mm.	0.149–0.074 mm.	Less than 0.074 mm.
	(%)							
A. Instant skim-milk powder	0.2	2.3	17.7	27.7	24.6	11.6	13.6	2.2
B. Instant skim-milk powder	2.1	7.6	6.5	11.4	15.5	17.2	32.4	7.0
C. Instant skim-milk powder				0.2	9.3	49.7	31.3	9.3
G. Agglomerated whole milk powder	1.1	8.3	11.1	25.1	28.4	18.8	7.0	0.2

TABLE 3

Effects of particle size on wettability and dispersibility

	Greater than 2.0 mm.		2.0–1.41 mm.		1.41–1.00 mm.		1.00–0.50 mm.		0.50–0.25 mm.		0.25–0.149 mm.		0.149–0.074 mm.		Less than 0.074 mm.	
Milk powder	Wet	Disp.	Wet	Disp.	Wet	Disp.	Wet	Disp.	Wet	Disp.	Wet	Disp.	Wet	Disp.	Wet	Disp.
	(%)															
A. Instant skim-milk powder	....	....	92	88	91	87	89	85	89	83	68	78	40	74	22	56
B. Instant skim-milk powder	91	82	90	80	90	80	88	80	87	78	78	76	47	60	24	42
C. Instant skim-milk powder	....	....	....	....	....	....	....	....	87	78	58	77	39	63	17	37
G. Agglomerated whole milk powder	7.8	....	6.5	7.5	5.8	6.0	4.7	5.3	4.2	5.1	1.9	4.6	1.5	9.9		

wettability and dispersibility. It will be observed that the wettability and dispersibility of the various fractions of skimmilk powder and of whole milk powder decrease with the decrease of particle size of the powder. The only exception to this may be found in the results which were obtained with agglomerated whole milk powder of particle size 0.149–0.074 mm. Decrease in particle size seemed to have a greater effect on wettability than on dispersibility. The low wettability of Product C may be attributed to the low average particle size of this powder. Although Product B has a higher percentage of large particles (2.0–1.41 mm.) than Product A, it also has a much higher percentage of small particles (less than 0.25 mm.) and a lower percentage of medium-sized particles. The agglomerated whole milk powder, produced in France, has a very low wettability and dispersibility at 25° C. However, at 40° C. the wettability and dispersibility were 92 and 70%, respectively.

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DETERMINATION OF BOVINE SERUM CALCIUM WITH A SIMPLE FLAME PHOTOMETER[1]

A large number of methods are available for the determination of serum calcium by flame photometry; however, these are fairly specific for the type of instrument used (1). Baird Associates, Inc. have distributed a tentative procedure for the determination of serum calcium, utilizing the Baird flame photometer.[2] While this procedure appeared advantageous in regard to time and ease of manipulation, no comparisons with accepted calcium methods were available. In principle, this tentative procedure is similar to several existing methods (1), in that anion interference is avoided through prior precipitation of calcium as the oxalate. However, the oxalate ion, at the concentrations which occur with this procedure, interferes in the flame photometric determination of calcium (1); therefore, the tentative procedure was modified to include oxalate in the standards.

Serum samples obtained from 86 different calves and cows were employed in this study during the period from December, 1957, to May, 1958. The standard method selected for comparison with the flame photometric procedure was that of Clark and Collip, as outlined in Hawk, Oser, and Summerson (2). As suggested, the samples were allowed to precipitate overnight. The procedure used in the flame photometric method was as follows: Five milliliters of distilled H_2O and 2.5 ml. of 3.0% ammonium oxalate solution were added to 5.0 ml. of serum in a 50-ml. conical centrifuge tube. The tube was stoppered, shaken, and allowed to stand 20 min. The tube was then centrifuged for 10 min. at 3,000 r.p.m. and the supernatant decanted. Five milliliters of 3.0% ammonium oxalate were blown directly on to the precipitate from a blow-out pipette and the tube recentrifuged for 5 min. The supernatant was again decanted, and the tubes were allowed to drain upside down for about 15 min. on filter paper. Three to four drops of concentrated HNO_3 were added and the tube shaken to bring the precipitate into solution. Four and three-quarters milliliters of 50 γ lithium per milliliter of solution were added and the tube swirled to facilitate mixing. Calcium concentration was then determined by measurement in the flame photometer, which had been standardized with solutions containing either 50, 100, or 150 γ calcium, 50 γ lithium, 200 γ oxalic acid, and 0.01 ml. HNO_3 per milliliter. For each set of determinations, the instrument was standardized with the 150 γ calcium per milliliter of solution. Readings were taken on the 100 γ and 50 γ calcium per milliliter standards and a constant, K, was calculated for each set of determinations as follows:

$$K = \frac{\text{Balance reading of standard}}{\gamma \text{ calcium per milliliter of standard}}$$

From the three K values, an average K, $K_{av.}$, was obtained and used to calculate sample calcium concentration as follows:

$$\gamma \text{ calcium per milliliter of sample} = \frac{\text{Balance reading of sample}}{K_{av.}}$$

[1] The author is indebted to H. D. Eaton for helpful suggestions in the preparation of the manuscript, and to G. S. Meyers, Jr. and D. G. Hazzard for technical assistance.

[2] The details of this tentative procedure were developed by J. U. White of The White Development Corp., and Sister Mary Assisum of St. Joseph's Hospital in Stamford, Conn.

Over a long period of time, which included making new standards, the K_{ac} value was found to be quite consistent. Statistical analysis of the data was carried out according to procedures described by Snedecor (6) and Lark (3). No appreciable differences existed between the range of values or between duplicates for calves or cows; therefore, these were not included as variables in the subsequent reporting of the data.

The mean value obtained by the Clark-Collip procedure was 10.81 mg. of calcium per 100 ml. of serum (range 8.04 to 12.26) and by the flame photometric procedure 10.18 (range 7.66 to 12.22). Thus, the value obtained by the flame photometric method was observed to be 0.63 mg. Ca^{++}/100 ml. lower than the mean Clark-Collip value. The basic errors in the Clark-Collip procedure appear to be incomplete precipitation of calcium and coprecipitation of magnesium as oxalate (4, 5). Both methods suffer from the possibility of incomplete calcium precipitation; however, coprecipitated magnesium oxalate would contribute to the apparent calcium content in the Clark-Collip procedure, but not in the flame photometric method. MacIntyre (4) examined the oxalate precipitates from ten serum samples, and found a range of from 0.0 to 11.0% magnesium coprecipitation. From this, it appears likely that the mean difference between the two methods was due to coprecipitation of magnesium oxalate in the Clark-Collip procedure. To test whether the differences in the means between the two methods were related to concentration of calcium in the serum, a linear regression of the average between duplicates of the Clark-Collip method minus those of the flame photometric method on the average of the Clark-Collip method was calculated (3). The intercept for this regression with its standard error was $+0.12 \pm 0.52$ and the slope $+0.05 \pm 0.19$, indicating that the average difference between means of the two methods was reasonably consistent over the range of calcium concentrations studied. The variance of the duplicates for the flame photometric procedure was almost double that for the Clark-Collip procedure, 0.0642 versus 0.0356. The tentative procedure distributed by Baird Associates, Inc. states that biological calcium determinations can be performed with an average difference between duplicates, expressed as a per cent of the mean concentration, of $\pm$ 5%. In this study, the corresponding value was $\pm$ 2.42%.

Preliminary recovery studies were carried out with pure calcium solutions to determine whether the precipitation, decantation, and other steps involved were of a quantitative nature. Sixteen determinations were performed, with solutions ranging in concentration from 80 to 120 γ calcium per milliliter, which is equivalent to 8–12 mg. of calcium per 100 ml. of serum. The mean recovery of calcium was 99.57%, with a standard error of $\pm$ 0.40, indicating that no appreciable loss was being incurred in the procedure.

The length of time proposed for complete precipitation of calcium from serum ranges from 30 min. to overnight (2, 5), depending upon the particular procedures consulted. With the aim of decreasing the precipitation time, and thus the over-all analysis time, an investigation of this point was carried out. Eleven samples of the same serum were set up for calcium analysis, and one tube analyzed after each of the following intervals: 5, 10, 15, 20, 25, 30, 35, 40, 80, 160, and 240 min. Plotting calcium concentration versus time for four such analyses, representing four different serum samples, indicated that the minimum precipitation time was between 12 and 15 min. On the basis of this, a precipitation time of 20 min. is suggested in the proposed procedure.

The results of this study indicate that flame photometric determinations of calcium by this modified procedure can be carried out on the Baird instrument. While this method gives results significantly lower than the Clark-Collip method, the difference is probably due to magnesium coprecipitation. In addition, recovery studies with pure calcium solutions indicated that this method was definitely of a quantitative nature. Though the duplicate variance for the flame photometric method was higher than for the Clark-Collip method, both variances are quite low (coefficients of variation were 1.74 and 2.48%, respectively, for the Clark-Collip and flame photometric methods), and the decrease in time and ease of manipulation are in favor of the flame photometric procedure. Preparation of serum samples for both methods is quite similar up to the titration or flame photometric portion of the analysis; however, titration requires approximately twice as much time as the flame photometer. The more samples being analyzed, the greater the amount of time saved with the flame photometric determination. This flame photometric determination would also appear to be advantageous in laboratories where serum calciums are run intermittently, since the standards are stable over a long period of time. With the Clark-Collip procedure, however, frequent restandardization of the potassium permanganate solution is required.

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EFFECT OF FEEDING N,N'-DIPHENYL-*PARA*-PHENYLENEDIAMINE (DPPD) TO LACTATING DAIRY COWS ON THE PRODUCTION OF BUTTERFAT[1]

Slight increases in the butterfat percentage were observed in three out of four experiments (3, 10, 11) when N,N'-diphenyl-*para*-phenylenediamine (DPPD) was included in the ration of lactating cows for the purpose of inhibiting copper-induced oxidized milk flavor. These results suggested that DPPD, when fed to lactating cows, may affect lipid metabolism. However, no definite conclusions could be drawn, since feed intakes were not rigidly controlled or a system of equalized feeding was not employed in the above-mentioned experiments. The present study was, therefore, initiated to determine the effect of feeding DPPD on the butterfat percentage of the milk of lactating dairy cows which were fed equivalent energy for maintenance and production. In addition, DPPD was determined in the milk.

Twelve cows, two Ayrshires, six Holsteins, and four Jerseys, were paired according to breed and insofar as possible according to age and days in lactation. Each cow of a pair was assigned randomly to one of two sequences of including DPPD in the ration, namely: DPPD—No DPPD—DPPD and No DPPD—DPPD—No DPPD, designated as Sequence A and Sequence B, respectively. All animals were fed a ration consisting of 21 lb. dairy ration (16% crude protein), 29 lb. dried beet pulp, and 50 lb. chopped, field-cured, second-cutting alfalfa hay per 100 lb. of ration. The animals were fed estimated net energy (ENE) at 105% of the upper Morrison requirements (7), based on milk production and maintenance needs observed at the start of the experiment. The animals were subjected to a 5-wk. standardizing period, followed by three 2-wk. comparison periods according to the sequences described above, with each comparison period preceded by a 2-wk. period during which only the basal ration was fed. Feed intakes were adjusted to new maintenance and production needs at the end of the 2nd wk. of the standardizing period. To limit the occurrence of weighbacks, the ration allowance was reduced to 100% of Morrison's requirements, based on previously observed maintenance and production needs 2 wk. prior to the start of the first comparison period. ENE of the feeds was calculated from the formula of Moore *et al.* (6) derived from Forbes's data, based on total digestible nutrient (TDN) values calculated from proximate analyses according to Schneider *et al.* (8, 9). DPPD (95% feed grade) was fed at a level of 0.01% of total ration (90% dry matter basis). The antioxidant was premixed with linseed oil meal and added daily to each cow's P.M. grain allowance during the comparison periods. Cows not receiving the DPPD were fed an equivalent amount of linseed oil meal. DPPD intake averaged 1.40 g. per cow per day, with a standard error of 0.07.

All feeds, fed and refused, and milk yield were weighed to the nearest 0.1 lb. twice daily. Live weights were recorded to the nearest pound on the sixth and seventh day of each weekly period.

Milk samples were taken twice daily during the 2nd wk. of the three comparison periods for butterfat determination by the Babcock method (1), and 4% fat corrected milk (FCM) was calculated according to Gaines (4). Samples of feeds were taken at each feeding and composited each weekly period for proximate analysis (1), Table 1. Weighbacks were collected during the 2nd wk. of each of the three comparison periods for proximate analysis. TDN content of the weighbacks was computed from proximate analyses according to the method of Schneider *et al.* (8) and ENE as previously described. Once prior to the feeding of DPPD, and once during each comparison period, samples of milk from cows receiving the antioxidant were obtained for DPPD determination. The concentration of DPPD in whole milk was determined quantitatively, following its separation from the nonsaponifiable portion of milk, by paper

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TABLE 1
Average proximate analysis of feeds and their estimated total digestible nutrient and estimated net energy content

Feed	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	TDN	ENE
	(%)					(lb.)[c]	(therms)[c]
Dairy ration	91.4[a] ± 0.1[b]	17.9 ± 0.3	56.0 ± 0.3	3.9 ±0.1	7.3 ± 0.1	73.7 ± 0.3	71.6 ± 0.3
Beet pulp	91.6 ± 0.1	8.3 ± 0.1	59.2 ± 0.3	0.5 ±0.1	19.2 ± 0.2	67.8 ± 0.1	63.1 ± 0.1
Alfalfa hay chopped	92.3 ± 0.1	16.1 ± 0.4	37.7 ± 0.8	1.8 ±0.1	29.8 ± 0.7	54.7 ± 0.5	43.7 ± 0.7

[a] Mean of 16 samples.
[b] Standard error.
[c] Per 100 lb. of feed.

chromatography. Fifty-milliliter samples of whole milk were lyophilized, extracted with 50-, 25-, and 20-ml. portions of peroxide-free ethyl ether. The supernatant was decanted after each extraction, centrifuged, and the combined ether extracts were evaporated under nitrogen to a volume of approximately 5 ml. and then saponified with 2 ml. of saturated alcoholic KOH for 15 min. on a steam bath under nitrogen. Fifteen milliliters of distilled water were added to the saponification mixture and, after cooling, the mixture was extracted twice with 20 ml. of peroxide-free ethyl ether. The combined ether extracts were then washed four times with 25 ml. distilled H_2O, evaporated to dryness under nitrogen, and the resulting nonsaponifiable fraction was made up to a volume of 0.2 ml. with benzene. Separation of DPPD from other constituents in the nonsaponifiable fraction by paper chromatography, and subsequent estimation of its concentration, have been described previously (2).

The data of this double-reversal trial were analyzed as outlined by Lucas (5). Data for only the last seven days of each comparison period were used in the statistical analysis. One Jersey cow developed viral pneumonia and had to be removed from the experiment.

Table 2 lists the treatment means, based on average daily responses observed during the last week of each comparison period, for the various milk production criteria. No discernible responses were observed for the supplementation with DPPD in pounds of milk produced, the percentage butterfat, or the daily yield of pounds of fat and FCM. These results suggested, under conditions of short-time feeding, that a level of 0.01% DPPD does not alter the lipid metabolism of lactating dairy cows as measured by the butterfat percentage of the milk. The level of feeding DPPD, 0.01% of 90% dry matter ration, was chosen on the basis of consistent inhibition of copper-induced oxidized flavor (3, 10, 11) and, therefore, higher levels of feeding were not considered in this study. No ready explanation can be given why under ad libitum feeding conditions, as employed in the previous studies, small increases in the butterfat percentage were observed in three out of four trials. One possible reason may have been increased feed intake, but this was not determined in the previous studies (3, 10, 11).

TABLE 2
Effect of feeding DPPD on various milk production criteria, energy intake, and utilization

Criterion	Ration		Standard deviation per animal[a]
	No DPPD	With DPPD	
Milk *(lb.)*	27.5	27.1	0.9
Fat *(%)*	4.4	4.4	0.2
Fat *(lb.)*	1.15	1.16	0.05
FCM *(lb.)*	28.3	28.2	1.1
Live weight (av. daily change, *lb.*)	+0.35	+0.25	1.01
TDN fed/day *(lb.)*	21.3	21.2	0.2
ENE fed/day *(therms)*	18.9	18.8	0.3
Nutrient utilization above maintenance			
Lb. TDN/lb FCM	0.52	0.52	0.03
Therms ENE/lb FCM	0.44	0.44	0.03

[a] Equivalent to the square root of the error mean square from the analysis of variance, and represents the differences in the quadratic trend of periods among cows within sequences. The standard error of the mean difference between No DPPD and With DPPD can be obtained by multiplying the standard deviation by

$$\sqrt{2\left[\frac{3(5+6)}{16(5)(6)}\right]}.$$

Average daily intake of estimated TDN and ENE were calculated, using each cow's actual feed intake and feed refusal and the proximate analyses for the particular week. As was expected, these values were similar between rations. To explore further the effect of feeding of DPPD, the efficiency of nutrient utilization for milk production was calculated. From individual energy intakes the maintenance requirement was deducted, employing 6.0 therms ENE and 7.0 lb. TDN per 1,000 lb.

live weight to the 0.67 power. The therms of ENE and pounds TDN required per pound of FCM were derived (Table 2) and found to be the same, whether DPPD was included in the ration or not. The relatively high values of 0.52 lb. TDN and 0.44 therms ENE per pound of FCM (7) can, in part, be explained by the fact that the animals gained weight during each feeding period. It was realized that calculations which considered live weight change probably would have given a more exact estimate of nutrient utilization. However, the short periods used in this experiment, and the small differences in live weight increases between DPPD and no DPPD, did not seem to warrant the added calculations.

Concentration of DPPD averaged 12.7 γ/100 ml. of milk with a standard error of 1.5, based on duplicate analyses of milks obtained from cows fed DPPD, and on a per-gram of fat basis 3.30 $\gamma \pm 0.37$. This resulted in an apparent mammary transfer of 0.14% $\pm$ 0.02 of the total DPPD fed. This value for mammary transfer was approximately four times as great as previously reported (11). It may possibly be a reflection of the quantitative method used in this study, as contrasted to the semiquantitative method used previously (11), and/or of greater efficiency of mammary transfer at the 0.01% level in this study as contrasted to the 0.10% level in the previous study.

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DEVICE FOR MEASURING RATE OF MILK FLOW [1]

The rate at which cow's milk flows has been investigated by a number of workers, using a variety of equipment to obtain and record observations (1, 2, 3, 4, 5, 6, 7, 8). An apparatus has been developed in this laboratory and found to perform exceptionally well as a means of recording the milking profile. It is essentially a continuous-feed kymograph (Figure 1), a self-contained, enclosed unit with an opening on one side through which notations can be made on the record. The weighing mechanism consists of a spring with a known stretch coefficient. In our studies we have used springs with stretch coefficients of 7 or 10 lb. per inch of expansion. The stylus is attached to a unit which operates on ball bearings to reduce friction. A steel clip extends from the unit and rests on a tubular collar fitted over and extending the full length of the weighing spring. The tube and collar unit may be adjusted to place the stylus at zero point, regardless of the tare weight involved. The driving

[1] Authorized for publication on June 11, 1958, as Paper No. 2267 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

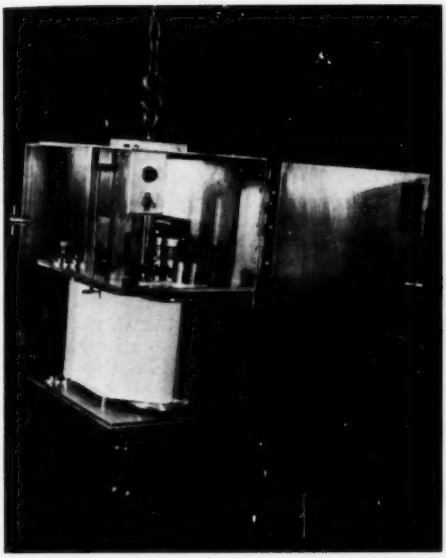

FIG. 1. Continuous feed kymograph suspended in the laboratory, showing internal structure. Feed reel is shown with the paper running under the stylus and beneath the black rubber roller which assures positive contact with the drive spindle.

mechanism is a synchronous motor geared to a positive-drive spindle. This spindle is fitted with two rows of stainless steel spikes, each 1/16th in. long, which pull the kymograph paper from the supply roll and across the stylus at a constant rate of 1 in. per minute.

With a time-weight relationship established, the milking profile can be read directly by using a transparent plastic grid, showing the yield of milk for each minute of milking time. Using as the zero point the origin of the coordinates, radiating lines are drawn on the grid which represent various rates of milk yield per minute.

In the milking parlor, the milk-receiving jar is attached to the kymograph, which is suspended from a 1-in. pipe placed overhead. In the stall barns, the kymograph is suspended from a wire cable located over the withers of the animals. By means of a steel hook, the kymograph is supported above the cow's back and at eye-level of the operator. The milker pail is suspended from the kymograph with another steel hook, at a distance from the floor which permits free spring extension as the milk enters.

Milking profiles were obtained on 142 cows comprising the University herds and including representatives of five dairy breeds. These animals were milked at 11-13 or 10-14 hr. intervals, the shorter period occurring during daylight hours. Five A.M. and 5 P.M. milkings were recorded. These milkings were consecutive in most instances. Since the over-all or average milking rate is greatly influenced by the efficiency of the person doing the milking, only the maximum-rate segment of the milking profiles was used in this comparison. An over-all average maximum rate of 6.3 lb. per minute was noted, with a range of from 2.3 to 14.9 lb. per minute. Repeatability of all milkings as measured by intra-cow correlation was determined to be 0.904. When considering either A.M. or P.M. milkings separately, the repeatability was 0.933. Beck *et al.* (1) and Thoele (7) have cited repeatability figures for maximum rate equally as high.

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OUR INDUSTRY TODAY

CAN DAIRY CATTLE BE BRED FOR INCREASED FORAGE CONSUMPTION AND EFFICIENCY OF UTILIZATION?[1]

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In these symposia on forage quality and its evaluation, and forage utilization, much of the emphasis has been on the aspects of the problem related to the plant and to management—how to fertilize the crop, when to cut it, how to harvest it (by animal or machine), whether to store it and how to store it, and finally, how to evaluate it. The animal may be called upon at the harvesting step, or may be by-passed if the forage is to be stored. In the final step, also, the animal may be used in evaluating the quality of the forage, or again it may be by-passed in favor of a laboratory procedure which may be much quicker and cheaper, but possibly less accurate. When the animal is used for evaluating the forage, we are, of course, interested in an average value applicable in general to all animals. Variation among the animals is usually considered undesirable, a source of error. But it is this variation among animals in which we must be interested, if we want to consider the possibility of breeding cows for higher roughage intake and efficiency of utilization.

Should we be interested in increasing the roughage intake of cows? Roughages are usually lower-priced than concentrates, even when they have to be purchased. Furthermore, a much larger proportion of the roughages than concentrates are home-grown in some regions. Reid (25) has cited studies which show that when roughage alone is fed at increasing levels, there is no decline in the TDN value; whereas, with corn silage containing corn grain there is a decline in TDN, and with mixed rations containing large proportions of concentrates the decline in TDN with increased levels of feeding is quite pronounced. Therefore, supplying more of the nutrients required in the form of roughage would reduce the depression of TDN, due to the higher planes of feeding which are necessary for high production.

Some of the factors influencing the total roughage intake of cows are shown in the arrow diagram (Figure 1). First, we might refer to the feed and management aspect, with the type and quality of roughage at the top of the list and with frequency of feeding and the amount of grain as further causes of variation in intake. These important factors have been covered in other papers at this symposium.

The animal factors might be organized differently, but certainly one of the most important of these factors is the capacity of the cow, which may be further called "effective capacity," since the maximum may be reduced by several factors. Next, the intake will be lesser or greater in relation to the desire of the animal to fill that capacity—full or only partly full—and this might be defined as appetite.

Effective capacity is, in turn, determined mainly by the size of the animal, but may vary to some extent as a result of a fetus displacing part of the space, possibly the encroachment of fat, previous feeding history, and the rate of digestion and rate of passage of food.

But what controls appetite? It is dependent on the needs of the animal for nutrients to support tissue replacement, activity, body growth, fetal growth, and milk production—a need to "keep the hopper full." There also would seem to be something inherent in the animal which may make it satisfied with the minimum, or to make it want to eat until it has to stop. This is what is meant by "intensity factor." Weather characteristics such as temperature and humidity, particularly at the extremes, certainly influence intake. It also seems likely that body condition affects appetite. In a limited amount of data we have observed that cows receiving no grain reached their peak consumption of roughage about 4 to 6 wk. after calving; whereas, cows on medium and high grain did not reach their peak consumption until about the 9th wk., or later. The no-grain cows lost their stores of body fat much more quickly than those receiving grain. Could this difference in body condition be a partial explanation of the differences in apparent appetite?

Which of these factors differ in a permanent way among animals? Which of them are controlled to some extent by inheritance? Previous feeding history, particularly that during the growth of the heifer, undoubtedly has an effect on the body size and effective capacity of the animal, as well as other characters; some of this effect would be permanent for the life of the animal, some of it temporary. It has been shown adequately that the size of the animal at a given age, the rate of growth, and the level of milk production are influenced by heredity.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, the State University, New Brunswick.

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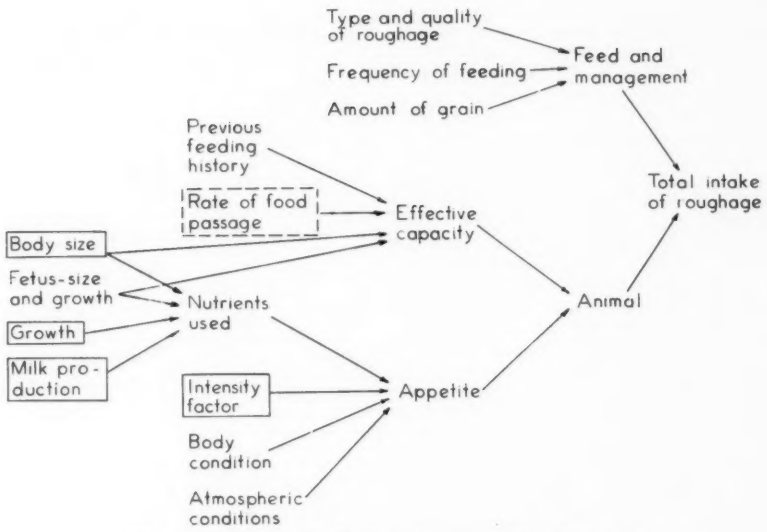

FIG. 1. Some factors affecting the total intake of roughage.

The intensity factor is probably a combination of hormonal and neural factors, both of which seem likely to be inherited to some extent. Comparatively little work has been done in measuring the rate of food passage through the digestive tract, but here also it seems possible that inheritance may play a part.

To answer the question "Can dairy cattle be bred for increased forage consumption?" we need answers to two principal questions (1) Is there any evidence that it is influenced by inheritance? and (2) to what extent is it influenced by inheritance—how much of the normal variation is attributable to differences in the inheritance of the animals? In the study of heritability of milk and butterfat production, we eliminate a considerable part of the variation in records by correction for age and by eliminating much of the variation due to differences in management and levels of feeding by analyzing on an intra-herd basis. The estimates of the part of the variation attributable to heredity differ in line with the degree to which such nongenetic sources of variation are standardized or adjusted for. In the case of roughage consumption figures, we must also make adjustments to overcome the nongenetic sources of variation.

Since there are practically no studies reported that have dealt with the heritability of roughage consumption, we must deal largely with the repeatability of estimates of roughage consumption. Repeatability measures the extent to which an animal retains its position or rank in a group from time to time under varying conditions, or the fraction of the variation which is a permanent characteristic of the animal. It may be considered as a combination of the inherited characteristics and the permanent differences brought about by environment, such as early feeding history. Thus, the repeatability of roughage consumption may be taken as the upper limit of the heritability, but the fraction of the repeatability which is likely to be heritability can only be surmised in the case of roughage intake. For butterfat production, the repeatability of yearly records is accepted as about .4, whereas the heritability is probably between .10 and .30.

Repeatability of raw roughage consumption data, unadjusted for differences in body size, might be very high, since skeletal size is a very permanent characteristic and there is usually a high correlation between body size and roughage consumption. In one study with 33 heifers, we found a correlation of .87 between body weight and consumption. Another group of 33 heifers gave a correlation of .90. Milking cows on three levels of grain feeding, but on an among-animal-within-year-ration basis, gave a correlation of .81 (29 degrees of freedom). However, Legates *et al.* (18) reported a correlation between body weight and intake at the peak of consumption of only .39 from one year to the next on the same animal. In a study of ten first-calf heifers, we found an among-animal correlation of only .48 of 2-wk. consumption at the peak. These ten animals were of rather widely differing body condition and level of production. These correlations would indicate the clear necessity of making some adjustment for differences in body size of animals.

Some consideration needs to be given to the method of adjusting for differences in body size. Assuming animals of equal body condition, the most simple adjustment is to cal-

culate the roughage per 100 lb. or per 1,000 lb. of body weight, and this is a commonly used method. However, this has a tendency to over-correct, so that small cows are credited with even better consumption than large cows. This was shown by Legates *et al.* (18) and in some of our data discussed in a paper at this meeting (22). This ratio assumes that intake is proportional to $W^{1.0}$. Kleiber suggested several years ago (17) that relative feed capacity should be calculated in proportion to $W^{3/4}$. We found, by actual fitting, that roughage intake of milking cows was proportional to $W^{.62}$ but, because of the limited range of size and the amount of error variation, we felt that the power .73 found by Brody for physiological body size was probably more appropriate (5).

The problem of different body condition and its influence on body weight is another problem to be considered, since the skeletal size of a 1,200-lb. thin cow is not the same as the skeletal size of a 1,200-lb. fat cow, and it can be assumed that the capacity of the digestive tract is more closely related to skeletal size than to body weight, which may vary with condition. Some skeletal measurement or combination of skeletal measurements would, therefore, have advantages over body weight as a base. Brody suggested the use of height at withers as a base for evaluating body condition. In our studies with heifers, the correlation of intake with width of hips was almost as high as with body weight and was higher than any of the other measurements, though the differences were very small. One of the reasons for the high correlation may have been the high repeatability of this measurement.

Referring to the arrow diagram (Figure 1), it would be easy to see that if the differences in intake are standardized with regard to feeding practices, and are made independent of effects of body size through some adjustment, and if measurements are made at stages of gestation before the fetus becomes very large, the major part of the influence of effective capacity would have been removed, and any variation remaining would be attributable to differences in the rate of food passage or to appetite. For the present, it will be necessary to consider the latter two together.

Huston (16) has accumulated roughage consumption data from several published studies and analyzed them on the basis of consumption per 1,000-lb. body weight, among cows within station and among years in cows. If the monthly variations were also included, combining another analysis of Huston's, the values in Table 1 were obtained. From these estimated repeatabilities, it may seem that if the consumption of one animal is measured for 1 mo. and compared with a 1-mo. value from some other year, the repeatability would be only .22; whereas, comparison of values within a lactation would give a repeatability of .54. Similarly, the comparison of yearly values would give a repeatability of .37. In this accumulation, the change in quality of roughage from one year to the next was not taken into consideration.

TABLE 1

Repeatability of roughage consumption per 1,000 lb., combining several sets of data from the literature [a]

Source	df[b]	M.S.[b]	V[b]
Cows in station (C)	32	322.0**	6.45
Records in cow (R)	47	131.3**	9.80
Months in record (M)	418	13.7	13.74

Repeatabilities:

Monthly values from any year—C/(C+R+M) = 0.22.

Monthly values in the same year—(C+R)/(C+R+M) = 0.54.

Yearly averages—C/(C+R+M/12) = 0.37.

[a] Data accumulated by Huston (16). Added calculations by the author. No consideration of change in roughage quality from year to year.

[b] df = degrees of freedom, M.S. = mean square, V = variance component.

An analysis of the data of Carrol (7) on seven cows for 2 yr. with three periods of 3 or 4 wk., in which the average effect of the differences in hay was removed, gave a repeatability of .66, which is markedly higher. If the average year difference was not removed, this repeatability was reduced from .66 to .34, showing the importance of removing the effects of changing roughage quality. In this particular case, the change was extreme, with an average consumption per 1,000 lb. of 33 lb. per cow in the 1st yr. and 23 lb. in the 2nd yr.

Our analysis of Iowa data (1) gave a repeatability of roughage $DM/W^{.73}$ of .32, based on 6-wk. periods with 18 cows and three rations.

Weekly consumption data from New Jersey for three winter feeding periods were analyzed to give more information on this subject (26). Cows received one of three rations, U.S. No. 1 extra leafy alfalfa hay plus corn silage with no grain, with grain at 1:6, or with grain at 1:3. Four breeds were involved. The pertinent data are given in Table 2. Animal variances were calculated on the deviations from means of the breed-ration subclasses, so that the effects of both breed and ration were eliminated. Unfortunately, only seven cows had data available for the full feeding period in 2 yr., so the year-in-animal term is subject to greater error than desirable. In this analysis, the average effect of years was not removed, but the difference in quality of the roughage was very small and not statistically significant. All of the variances in this table are less than in the study of published data and the repeatability of 3-wk. averages of .52 from any year is considerably higher than the comparable value of .22. If adjusted for the average year difference, the repeatability of 3-wk. averages was increased to .60. In these analyses the adjustment for

TABLE 2

Repeatability of roughage dry matter intake adjusted for weight by covariance—hay study [a]

Source	df[b]	M.S.[b]	V[b]
Animal in breed-ration (A)	19	119.62**	3.96
Year in animal (Y)	6	24.81**	0.83
Weeks in year in animal (W)	707	8.55	8.55

Repeatabilities:

Weekly averages from any year—
$A/(A+Y+W)=0.30$.
3-week averages from any year—
$A/(A+Y+W/3)=0.52$.

[a] Makes no allowance for roughage change from year to year or decline in intake during the feeding period.

[b] df = degrees of freedom, M.S. = mean square, V = variance component.

size was by the among-animal regression on body weight.

An experiment using the same grain feeding levels, but with grass silage fed to capacity in addition to 5 lb. alfalfa hay, was also analyzed. In this case the 3-wk. peak consumption values were used. In this analysis the cows were in the study for 2 or 3 yr. The year-to-year repeatability from this study was .55, with adjustment for body weight and grain level by covariance (12 degrees of freedom for animals, 30 degrees of freedom for years in animal).

In the last two winters we have had 33 heifers between the ages of 15 and 32 mo. on trial, to determine roughage intake. Since we had only 11 stalls available, the heifers were rotated through the stalls, in groups of 11 three times, or cycles. The heifers were in the stalls for 2 wk. with the first four days for adjustment and the remaining ten days divided into two periods of five days each. A series of body measurements was made during each five-day period. A constant amount of grain was fed to all heifers, 1.5 lb. per day. The repeatabilities are given in Table 3. The values for 1958 are somewhat higher than those for 1957. The values for 1957 are based on total dry matter intake; those for 1958 are for both total dry matter and roughage dry matter intake.

All of these studies would seem to indicate that there are real differences in the appetite of dairy animals as measured by total roughage intake adjusted for body size. The various repeatabilities are summarized in Table 4. With a normal range of animals, the repeatability of intake is probably about .5, or higher, when based on estimates of 3 to 4 wk. This may be compared with the .4 repeatability for yearly butterfat records.

Will the values obtained as heifers represent the animals well after they have calved? With only ten of the 1st-yr. heifers we found that the repeatability from heifer to cow was only about half as large as among comparable periods as heifers, indicating that it would be best to select animals on the basis of intake after calving. Other considerations indicate that it is probably better to determine intake on the same type and quality of roughage

TABLE 3

Repeatability of total dry matter intake or roughage dry matter intake of dairy heifers with adjustment for weight by covariance and by the ratio to $W^{.73}$

	1957[a]		1958[a]	1958[b]	
Means	Covariance	Ratio	Ratio	Covariance	Ratio
Periods (5 days)	.32	.30	.55	.46	.58
Cycles (10 days)	.38	.37	.66	.56	.68
3-cycles (30 days)	.65	.64	.85	.79	.90

[a] Total dry matter intake.
[b] Roughage dry matter intake.

TABLE 4

Summary of repeatabilities of roughage dry matter consumption for dairy cows and heifers

Source	Repeatability
Published works combined (16)—months in the same year	.54
Published works combined (16)—months in any year	.22
Carrol (7)—3- or 4-wk. periods—different cuttings of hay	.66
Autrey *et al.* (1)—6-wk. periods, three levels of grain, ratio adjustment	.32
New Jersey studies (26)	
Cows—hay experiment—3 wk.—covariance adjustment	.52
Cows—silage experiment—3 wk.—peak—covariance adjustment	.55
Heifers—1957—30 days—covariance adjustment	.65
Heifers—1957—30 days—ratio adjustment	.64
Heifers—1958—30 days—covariance adjustment	.79
Heifers—1958—30 days—ratio adjustment	.90

likely to be fed under normal conditions, since there was a rather high interaction between animal and hay when the changes in hay quality were marked.

We have found no published results of estimates of heritability of roughage intake when fed to capacity. In the two heifer studies referred to before, the paternal half-sister correlations were calculated, even though the data are very limited in scope. In the 1st yr. there were seven sires with two or more daughters each and a total of 20 daughters. In the 2nd yr., six sires were represented by two or more daughters, for a total of 30. Using the ratio of intake to $W^{.75}$, the correlations were .104 and .035 which, multiplied by four, give values of .42 and .14. Since these estimates are based on such small numbers, we would be dignifying the values unduly by calling them heritabilities, so we should merely say that there is limited evidence that inheritance probably plays a part in determining the appetite of dairy animals for roughage. There is more evidence for saying that animals do differ in their desire to consume roughage.

The second part of the topic refers to the efficiency of utilization of roughage. To be utilized for productive purposes, a feed must first be digested and the nutrients absorbed. In ruminants, a large part of the digestion takes place in the rumen through the work of microorganisms. Therefore, it might seem that there would be little opportunity for the animal itself to influence digestion in the rumen. However, the animal must provide suitable conditions for the organisms to work most effectively. Are there differences among animals in amounts and proportions of buffering and other substances in the saliva, in rumen motility, in the absorption of fatty acids through the wall of the rumen, in the blood flow to the rumen? On this last point the work of Conrad and coworkers at Ohio (10) is of interest. Using erythrocytes tagged with Phosphorus[32], blood flow to the rumen, reticulum, omasum, and part of the abomasum was estimated in nine calves. The blood flow in milliliters per minute per 100 lb. body weight varied from 365 to 878 ml.

What have been the results of actual digestion studies? We have made calculations to estimate the repeatabiliy of digestibility of various components of the feeds from several published studies and some unpublished data from New Jersey.

Repeatabilities of digestion coefficients calculated from five studies with sheep are presented in Table 5. Repeatabilities which are shown here as negative values are usually interpreted as meaning zero. However, since averages were calculated, the negative values found are presented. Since variances from the various studies were quite variable, the more refined methods of averaging did not seem justified, so the simple averages of the correlations

TABLE 5

Repeatabilities of digestion coefficients calculated from published data and unpublished data from New Jersey

	Reference	No. animals	Observations/animal	Repeatability				
				D.M.	Protein	Fiber	NFE	Energy
Sheep								
1	23	8	4		.11			
2	29	5	2	.38	.41	.11	.62	.31[a]
3	13	5	2	.37	.82	.11	−.48	.33
4	20	4	4	.37	.61	−.21		
5	3	8	4	.38	.09	−.01		.39
Steers								
6	33	4	4	.57	.09	.34	.74	
7	33	6	6	.39	.14	.23	.34	
8	29	5	2	.66	.22	.35	−.24	.57[a]
9	30	6	6	.02	.20			
10	31	6	6	.41	.10			
11	32	6	6	−.01	−.09	.07	.00	.01
12	9	4	3	.02	−.04	.38	−.27	−.23
13	2	10	2	.75		.35		
14	24	4	12	.50		.31		
Heifers								
15	8	8	5	.21	.29	.37	.03	.24
Milking cows								
16	11	10	3	.52	.23	.29	.58	.41
17	19	4	4	.03				
18	4	36	6[c]	.42	.65	.53	.52	
All sheep[b]		30		.37	.41	.00	.07	.34
All cattle[b]		109		.35	.18	.32	.21	.20

[a] TDN.
[b] Simple means of observed repeatabilities.
[c] Three days, two collections per day.

are given in the second last line. Considerable variability is indicated for some components. On digestiblity of energy or calculated TDN and dry matter, these sheep data are fairly consistent and show that about 35% of the animal-plus-error variability was attributable to animal differences.

Similar calculations were made on several studies with cattle, some beef, some dairy, with the results also given in Table 5. Again, there is considerable variability with some negative values appearing. However, the mean repeatability for the digestibility of dry matter is very close to that for sheep. A marked difference is noted on the digestibility of crude fiber, however, the average for sheep having been zero and this one for cattle being .32, with no negative values found.

In most digestion studies with steers and with sheep an attempt is made to use animals that seem uniform. However, in Study No. 16, the ten dairy cows were of three breeds—Holstein, Guernsey, and Jersey. In Study No. 18, the 36 Holstein cows covered a wide range of producing abilities. The figures in this table indicate that there are real differences in the digestive capacity of ruminants.

After nutrients are digested and absorbed, they must be converted into energy for maintenance, body growth, or milk production. Forbes *et al.* (12) showed that metabolizable energy was used for milk production 98.5% as efficiently as for maintenance, but for body gain only 76% as efficiently. Thus, it is readily seen that the cow which converts its feed to milk is using energy more efficiently than the one which converts some to milk and some to body fat. We know that cows differ greatly in the level at which they will begin to put extra energy into body fat instead of into milk.

As example of the different responses of cows of different milk-producing abilities comes from the New Jersey study previously referred to. Three levels of grain were fed in addition to grass silage to capacity with limited hay. Six groups of Holsteins differed in 245-day milk-producing potential, estimated from previous records, from about 9,000 to 12,000 lb. FCM. In the lowest group, for each pound of grain fed, 0.58 lb. extra FCM was produced, comparing the 1 : 3 ration with the all-roughage ration. The highest potential group gave an extra 1.40 lb. extra FCM per pound of grain, about 2.5 times as much. The other groups were intermediate, and there was a correlation of .88 between potential and response to grain. On an ENE basis the differences between groups were even greater.

Huston (16) calculated repeatability of the gross efficiency figures computed by Brody on the data of Harrison and Savage (1932) and found .47.

In the Iowa study (1), the repeatability of net efficiency (nutrients above manitenance) was only .03.

Three recent studies on the efficiency data from the Danish testing stations are available. Expressed as total feed units per kilogram butterfat, the heritability of efficiency was .4, but the net efficiency of production feed units (above maintenance) per kilogram butterfat gave a heritability of only .2 [Venge (28)]. Gjelstad used FCM/total feed units consumed (gross efficiency) and found a heritability of .39 (14). Mason *et al.* (21), using this same formula, found a heritability of .48. Thus, it is quite apparent that the answer to the question of whether efficiency is inherited is "Yes."

Thomas and Moore (27) recently showed that there was a statistically significant difference among 11 cows in the amount of dry matter needed to maintain body weight. They estimated the repeatability of the measurements to be .41. Thus, the efficiency of the cow varies not only in regard to milk production above maintenance requirements but also in regard to maintenance itself.

At this meeting, Hooven and Matthews (15) have just reported that the repeatability of gross efficiency was .34. They also found that daughters of six sires differed significantly in efficiency, even after adjustment for level of production and body weight differences; they did not present a heritability estimate.

The question involved in the title of this discussion: Can we breed cows for increased forage consumption and utilization? would seem to be answerable in the affirmative. But we might also ask, Should we? Venge (28) estimated that the feed saved in feeding 20 heifers above average in efficiency, compared with 20 below average, could save enough feed to keep two more cows and produce 310 kg., or 860 lb., butterfat.

Swine breeders and poultry breeders have been giving consideration to efficiency of feed utilization for quite a while now and have accomplished great things in improved efficiency. Why haven't dairy cattle breeders been considering it? The principal answer lies in the very high correlation between efficiency and level of milk production. When we select for milk production we are also selecting to a large extent for gross efficiency. Mason *et al.* (21) found a genetic correlation between gross efficiency and milk production of .95.

Kleiber (17) has reasoned that one of the most important considerations in selecting for efficiency is to consider relative feed capacity, which is the ratio of intake to $W^{3/4}$. Thus, with selection both for high milk production and for high feed consumption, especially of good-quality roughages, we should be selecting automatically for increased gross efficiency. Selection for either would also tend to increase efficiency, but the genetic correlation between the two has not yet been determined, to indicate whether selection for one factor would be almost as effective as selection for both.

From the practical standpoint, how should we go about selecting for high roughage intake? Measuring roughage consumption for all the cows over a long enough period of time to get a good and repeatable estimate of the inherent capacity of the cows of a herd would be quite impractical for the farmer. One of our objectives should, therefore, be to find other methods of getting good estimates of the capacity of the cow. Burt (6) has measured the amount of feeds eaten in 60 min. Perhaps some such measure might be tried to advantage, to get faster results.

In summary, measurements of roughage intake are repeatable to the extent of .5 or more for periods of about 3 wk. or a month, and are probably inherited, but further work is needed on this point. Efficiency is also inherited, but selection for high levels of milk production and high rates of roughage intake also would result in increased efficiency. Greater efforts should be directed toward a better method of evaluating the total roughage intake of dairy cows.

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EFFECTIVENESS OF VARIOUS SYSTEMS OF FORAGE UTILIZATION IN THE SOUTH[1]

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It is the objective of this paper to compare the alternative pasture systems used in the South, with special emphasis on soiling. The various problems connected with the forage utilization methods as indicated by various dairy department personnel in the southern region will be mentioned.

The ultimate purpose of research on alternative pasture systems should be to present information that will enable the dairy farmer to select the method or combination of methods producing the greatest farm net income. It is obvious that we should obtain the highest milk production per cow per acre from forage. The problem is, Which method or combination of methods of pasture utilization produces the best results? The current demand for increased efficiency on the farm has made us more aware of these problems. According to Hoglund (8), "To be of value to the dairy farmer information should be made available to show how investment, labor requirements, expenses and net income are affected by various combinations of alternative pasture systems." To determine the relative value of the various systems in terms of net returns it is necessary to compare them on a basis of (1) milk production and body weight changes, (2) excess forage available for storage as hay or silage, and (3) fitting the systems into the over-all management of various farms.

In attempting to prepare a report such as this, many difficulties present themselves. First of all, a search of the available literature indicated that few research reports involving comparisons of soiling and other pasture systems were available. In this instance, personal communication was far more fruitful. But even when unpublished data were obtained, the problem of reporting was not solved, because of the following factors: 1. The wide variety of conditions under which the various experiments were carried out; 2. the different objectives involved; 3. the different measures of output used; 4. the differences in reporting of methods and results. In brief, a common denominator is needed.

Several states in the South have carried on research comparing the alternative pasture systems. The objectives of this research, to be considered here, fall into three categories. These are: 1. To compare soiling with grazing methods; 2. to compare soiling with stored forage and grazing methods; 3. to partition the direct and indirect effects of climate on plant and animal, using soiling and various grazing methods.

One of the objectives common to all the experiments was to determine the effect of systems on milk production Owen *et al.* (10) of Mississippi, Stone (12) of Louisiana, and Johnston *et al.* (9) of Louisiana all found soiling to be superior to some form of grazing and/or to stored forage in terms of daily milk production. Swanson (13) of Tennessee obtained essentially no difference between soiling and strip grazing. Ellzey (4) of Louisiana found that soiling produced significantly less milk per cow per day than the other systems. However, in this experiment the green chopped forage continued to mature throughout the experiment, while the other forage plots were grazed, regrowth appeared and was then utilized. This could be considered a weakness inherent in the system. This factor affects the outcome of the experiment, especially with a crop such as Pearl Millet. These results show the variation possible among experiments.

[1] Presented at the joint meeting of the American Grassland Council and the American Dairy Science Association at North Carolina State College, Raleigh, June, 1958.
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It is not a simple matter to rationalize for these variations, except to say they may be due to differences in animals and/or physical layout. For example, acre-cow ratios on experimental grazing plots can exert a definite effect on results, as can quality and quantity of available forage.

TABLE 1

Summary of effect of grazing method on milk production

Author	State	Grazing method		
		Soiling	Strip	Rotational
		(lb. 4% FCM per cow per day)		
Owen (10)	Mississippi	29.0	26.6	28.0
Ellzey (4)	Louisiana	20.8	25.2	26.6
Gordon (5)	USDA	27.5	25.8	27.5

Table 1 summarizes results of three of the experiments which compared soiling and strip and rotational grazing directly, and effects on milk production. It can be seen that in two instances rotational grazing was superior to strip grazing in terms of milk production. The work of Gordon *et al.* (5) shows soiling and rotational grazing to be of equal capability on the basis of FCM production per cow per day, whereas strip grazing was inferior to the two other methods. These results possibly may be explained in terms of the greater selectivity of forage normally possible under rotational as opposed to the more restricted strip grazing.

Although the work of Ellzey points out that on the basis of milk production per cow per day soiling was inferior, from the standpoint of milk per acre, soiling produced over 400 lb. of milk per acre more than rotational grazing. Thus, despite the fact that soiling produced less milk per cow, in one sense it was actually a more efficient method, for a given acre supported more cows and produced more total milk (4, 5, 10, 13). In cases where soiling and other grazing systems produced similar results on the basis of milk production per cow, the choice of systems depends upon other factors. Therefore, if land utilization is in favor of soiling, and increased costs are such that increased farm net income will be realized, then the system of choice becomes obvious.

Soiling has been shown to increase land utilization both experimentally and under various management conditions (1, 4, 7, 10, 11, 13). The work of Swanson (13) of Tennessee points out the increased land utilization possible under soiling compared to strip grazing. On the basis of milk production per acre Swanson, using alfalfa, obtained 123% more milk per acre from soiling. Comparing the same treatments using Sudan grass, a 17% increase in favor of soiling was realized. The significance of these comparisons is increased when the forage in excess of feeding needs, which was removed from the fields as silage, is considered. Alfalfa fields that were used for soiling yielded 3.87 tons of silage per acre, as compared to 1.12 tons per acre from the strip-grazing acreage. The Sudan grass fields used for soiling produced 2.53 tons of silage per acre as opposed to none from the strip-grazed fields.

While these results indicated a wide increase in land utilization in favor of soiling, the work of Owen *et al.* (10) (Appendix Table 2) shows soiling to yield an increase over strip and rotational grazing of 9 and 47%, respectively. The work of Ellzey (4) shows an increase in carrying capacity of 71% for soiling, both over rotational and over strip grazing, which had equal capabilities in carrying capacity.

The results of Gordon *et al.* are in opposition to the three above-mentioned experiments. Gordon and associates (5) (Appendix Table 3) obtained significantly different results between strip and rotational grazing and soiling, when results were measured in terms of TDN per acre and season. However, on the basis of 4% FCM production, soiling and rotational grazing were equal. The treatment differences in Gordon's work were not significantly different. This work showed that the nutrient requirements of the cows were met equally well on all systems, but that more acres were required to meet these needs under the soiling system. It is Gordon's opinion that the results of this experiment emphasize that the possibilities for increased carrying capacity from soiling are directly associated with the magnitude of grazing wastes and that grazing wastes, in turn, are closely related to the type of crop used and grazing management. It is my opinion that, in part, this statement is true. One must remember, however, that in comparing soiling with grazing methods, certain advantages are in favor of soiling; for example, the reduction of losses due to trampling and due to fouling of the forage by excreta. These factors must be included when comparing the relative value of the systems.

So far, only milk production and land utilization have been considered, but other points of significance, such as body weight changes and forage intake, deserve to be mentioned.

Body weight changes on the alternative pasture systems were not reported in all cases. Owen *et al.* (10) indicated that cows on zero grazing gained 30.5 lb., whereas those on rotational and strip grazing gained 10.7 and 7.4 lb. per cow, respectively, over a 68-day period. The work of Ellzey (4), making the same comparisons over a total of 60 days. showed that cows on zero grazing gained 23 lb., whereas those on rotational and strip grazing lost 13 and 21 lb., respectively. No apparent significant differences were observed in body weight of cows on Swanson's experiment.

In 1955, Stone *et al.* (12) (Appendix Table 4) of Louisiana conducted an experiment in an effort to compare soiling, silage, and rotational grazing on pasture, using a common crop, Pearl Millet, compared with alfalfa hay as the con-

trol. During the 40 days of the experiment, radical changes in proximate analyses of the green feed and grazing crop were observed. A 70% increase in dry matter, a 21% increase in crude fiber, and a 49% decrease in crude protein were observed. The change in dry matter composition indicates it is not valid to put emphasis on forage intake on a wet-weight basis.

The average daily dry matter intake for Period 1 was 28.7 ± 3.1 and for Period 4, 25.7 ± 2.5. While on a wet-weight basis, green chop intake varied from 152 to 80 lb. during the same period of time.

Mean differences indicate that millet soiling sustained greater 4% FCM production at a highly significant level (1%), as compared to other treatments. Differences between milk production from alfalfa hay and millet silage were significant at the 5% level, although differences between silage and pasture were not significant.

Alfalfa hay maintained body weight better than any other treatment, and differences between this and all other treatments were highly significant. Silage was superior to pasture in maintaining body weight at the 5% level of probability.

Certain environmental factors have governed the choice of alternative pasture systems used in the various climatic zones within the South. In the Gulf Coast region, weather is one of these factors. The work of Johnston *et al.* (9), using soiling and rotational pasture combined with shade and air conditioning, has attempted to partition the direct and indirect effects of climate on the lactating cow. Most recent studies indicate that nutritional factors probably are more important in maintaining summer milk production than the effect of thermal stress on the physiology of the cow. These particular studies also point out that a combination of soiling during the days, combined with pasturing at night, might be the management system of choice under such environmental conditions.

Table 2 shows the results of the most recent study by Johnston and associates, by treatments.

The foregoing discussion has indicated the value of the various systems for forage utilization as determined by research. Those who have conducted the work, and others who have used these alternative pasture methods as management practices, have contributed to the following through their concepts of the problems peculiar to the various systems.

TABLE 2

Summary of the effect of soiling and rotational pasture on animals under different environmental conditions

Forage supplied as:	Treatment	Daily milk production	Decline
		(lb. 4% FCM)	*(%)*
1. Soiling	Air conditioning	25.4	−3.7
2. Soiling	Shade	23.8	−6.2
3. Rotational pasture	Pasture	22.9	−6.7

One problem reported by several Southern workers was the difficulty encountered in maintaining high-quality forage for adequate periods during the summer (2, 4, 6, 9, 10, 12, 13). This difficulty primarily is associated with the use of temporary summer-pasture crops. Certain permanent pasture crops, Dallis Grass, for example, have not proved satisfactory in Louisiana as soiling crops, since the cattle refuse to eat them in adequate amounts. Little is known about nutrient variation and animal responses to these crops. Experience in Louisiana (9, 12) indicates that dry matter intakes appear to remain relatively constant up to the early reproductive stage of the plants. During exceptionally dry periods intake drops more rapidly. One possible explanation is that rapid lignification occurs, resulting in a decrease of cellulolytic microorganisms in the rumen and a subsequent decrease in appetite; hence, the reduction in dry matter intake.

Another problem primarily affecting the practice of soiling is that of excessive rainfall, which prohibits getting into the fields with equipment. In the Mississippi experiment of Owen *et al.* (10), rain would not permit forage chopping for 19 days, or 28% of the experimental period, during which time alfalfa hay was substituted for green chopped Sudan grass. Ellzey (4) of Louisiana encountered the same problem for 25% of the entire experimental period, which forced him to cut the millet forage by hand and feed it through the forage harvester. Ragsdale (11) reports that work in Missouri demonstrates the possibility of substituting good-quality hay for green chop for short periods without significant changes in milk production.

Swanson (13) of the Tennessee Dairy Department, and Chamblee (3), North Carolina agronomist, have suggested that chopping various crops might have an effect on crop recovery and maintenance of the crop stand. It was Swanson's observation that chopping alfalfa caused slower recovery of the crop and that summer droughts more seriously affected the chopped alfalfa than that which was grazed. It was suggested that future research be conducted on the effect of chopping versus grazing on the physiology of various crops cut at various stages of maturity and at different plant heights (3, 13).

The advantages and disadvantages of soiling as compared to other systems of forage utilization may be summarized as follows:

I. Advantages of soiling:
   - A. Increase forage yield per acre by decreasing losses due to trampling, manure contamination, and uneven grazing.
   - B. Make possible the use of distant fields not readily accessible for grazing.
   - C. Make for more uniform day-night for-

age intake than grazing when fed in the shade during hot weather.

D. Make for more efficient use of machinery—excess forage can be ensiled at any time.

E. Prolong the period in which fresh green forage may be fed from a given field.

F. Provide an excellent research tool.

II. Disadvantages of soiling:

A. Limit the practice to larger herds because of equipment costs.

B. Create manure problem, since manure either is lost or must be collected and returned to the field mechanically. This could be an advantage.

C. Eliminate selectivity of the cow—it must eat the whole plant rather than selecting just the more nutritious parts.

D. Use subject to topographical limitations.

III. Soiling is mainly dependent upon:

A. The availability of harvesting equipment.

B. The cost factor of soiling being less than income derived from its use.

C. The availability of ecologically suited species of crops lending themselves to quality and quantity of production and whose growth habits are not too radical or erratic.

D. A supply of supplementary stored forage which would:

1. Help to eliminate day-to-day variation in milk production.
2. Be available during periods when it is impossible to get on the field with equipment.
3. Provide for situations when the quality and quantity of forage soiling crop is insufficient.

In summary, it appears that no one system of forage utilization is best for all situations. The problem of which system of forage utilization to use is largely dependent upon local conditions. The farmer must decide which combination he will use, based on principles of good dairying, economics, and sound logic, as determined by the capabilities of himself and his farm.

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APPENDIX TABLE 1

Methods of feeding Pearl Millet to lactating dairy cattle

H. D. Ellzey

Louisiana Agricultural Experiment Station, Franklinton

		Rotational grazing	Strip grazing	Green feeding
Acres required per cow	Period 1	.49	.49	.38
	Period 2	.67	.67	.40
Average milk production	Period 1	28.7	27.7	22.8
	Period 2	24.5	22.6	18.7
Total pounds milk per cow	Period 1	861.0	831.0	634.0
	Period 2	735.0	678.0	561.0
Total pounds milk per acre	Period 1	1,757.0	1,696.0	1,800.0
	Period 2	1,096.0	1,012.0	1,400.0
Body weight change	Period 1	−23	−32	−10
	Period 2	+10	+ 9	+33

Average consumption by ten-day periods, green weight for soiling group

	First 10 days	Second 10 days	Third 10 days	Average
Period 1	146	146	118	136.6
Period 2	139	108	113	120.0

APPENDIX TABLE 2

Summer pasture studies with dairy cattle

J. R. Owen, W. C. Cowsert, and J. T. Miles

Mississippi Agricultural Experiment Station, Starksville

State	Grazing system	4% FCM milk production[a]	Cow-days grazing	Dry matter consumption	Weight change	Length of experimental period	Persistency index
						(days)	*(per cent decline)*
Mississippi	Rotational	28.0	111	.....	+10.7	68	23.1
	Strip	26.6	149	.....	+ 7.4	68	26.2
	Zero	29.0	163	20.6	+30.5	68	20.2

[a] Differences among treatments not significant.

APPENDIX TABLE 3

Unpublished data

Gordon *et al.*, USDA,[a] Dairy Research Branch

Summary (based on per year per acre)

		Rotational grazing	Strip grazing	Soiling
1.	Cow days grazed	133	135	103
2.	Grazed or fed green per season *(lb. TDN)*	2,216	2,110	1,802
3.	Per cow per day *(lb. TDN)*	16.7	15.6	17.5
4.	Harvested as excess *(lb. TDN)*	752	905	668
5.	Total pounds TDN	2,968[b]	3,015[c]	2,470[b,c]
6.	Total pounds FCM	4,227	4,061	4,277
7.	Av. FCM per cow per day	27.5	25.8	27.5

[a] Unpublished data provided through courtesy of Dr. Gordon, USDA.
[b] Difference statistically significant at the 5% level of probability.
[c] Difference statistically significant at the 5% level of probability.

APPENDIX TABLE 4

Comparison of pasture, hay, silage, and soilage as the sole roughage for lactating cows under subtropical conditions

Summary[a] of forage consumption, 4% milk production, and body weight changes, by treatments

Edward J. Stone, J. E. Johnston, and J. B. Frye, Jr.
Louisiana Experiment Station, Baton Rouge

	Millet soiling crop		Alfalfa hay		Millet silage		Millet pasture		L.S.D. .05	L.S.D. .01
	(Mean)	*(S.D.)*	*(Mean)*	*(S.D.)*	*(Mean)*	*(S.D.)*	*(Mean)*	*(S.D.)*		
Forage consumption	104.0	8.3	27.4	3.7	69.0	6.9	.....	.....		
Forage dry matter consumption	24.0	4.2	24.8	3.2	22.0	2.9	.....	.....	1.75	2.35
Milk production, 4% FCM	30.0	6.5	27.2	6.5	25.0	4.9	23.2	5.9	1.89	2.55
Body weight changes[b]	−9.5	14.0	+3.3	10	−6.8	9.0	−15.8	11	7.50	10.15
Dry matter digestibility *(%)*	51.9		51.9		47.3					
Ratio DM consumption: Milk production	1:1.25		1:1.10		1:1.13					

[a] All weights expressed in pounds.
[b] Expressed as period means.

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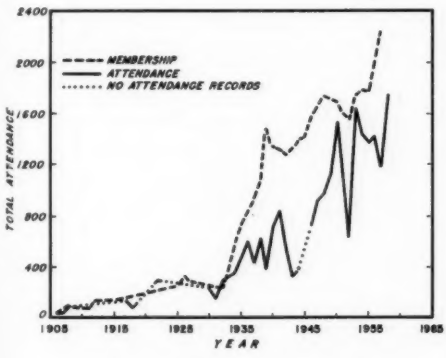
Michigan State University, East Lansing
Historian, American Dairy Science Association

The annual meeting of the American Dairy Science Association has become a mecca for its members, their wives, children, and friends. Attendance has been progressively upward during the past 30 years; this despite the Great Depression, and World War II with its gas-rationing. Attendance at the annual meetings of the American Dairy Science Association, for the most part, during the past 25 years has been very gratifying. The data presented in Table 1 and shown graphically in Figure 1 indicate that attendance at future meetings may be expected to be high, barring economic disaster and wars.

Analysis of attendance figures from time to time would seem to give perspective to the members and at the same time be of much value to those planning meals, lodging, and program-room accommodations. To this end, the observations reported herein were made.



Summer, College-Campus Meetings Furnished Impetus to Attendance

Inauguration of the annual summer meetings on college campuses was the beginning of other-than-routine interest in the annual meetings. At the 22nd annual meeting, held on the campus of the Michigan Agricultural College (Michigan State University, 1955), East Lansing, June 22-24, 1927, a few members dared to bring their wives. This move was viewed with no little concern. No special ladies' program was arranged. The women had to fend largely for themselves. To their credit, they neither interfered with nor attended the meetings. Only at the banquet and fish fry, the social portions of the program, were they in evidence. Their presence seemed to grace these affairs. Several members thought perhaps bringing wives to the meeting, thereby mixing the family vacation with business, was not a bad idea after all. Thus, the real beginning of a marked rise in attendance.

Yet, five years were to pass before another innovation crystallized, making changes in planning the programs necessary. In the very midst of the Great Depression, 1932, the hosts at the University of Kentucky, Lexington, with Fordyce Ely, chairman, showed a graciousness in entertainment of their A.D.S.A. guests, which since may have been equalled, but never surpassed. Here, the entertainment feature was introduced. Also, the preprinting of abstracts of papers presented indicated that no stone was left unturned to make the meeting a successful one. The pattern set by Kentucky has been followed more or less ever since, as conditions and facilities permit.

Naturally, such hospitality and entertainment, possibility of seeing new campuses and facilities, and renewal of acquaintances led one to look forward to the next meeting.

Yearly Attendance Constantly Increasing

From a nucleus of 17 in 1906, the yearly attendance has increased to a record high of 1,732 at North Carolina, 1958. During the life of the Association, attendance seemed to have peaked out at progressively higher points seven times. These were St. Paul, Minnesota, 1922; State College, Pennsylvania, 1936; Columbus, Ohio, 1938; Burlington, Vermont, 1941; Ithaca, New York, 1950; Madison, Wisconsin, 1953; and Raleigh, North Carolina, 1958, with respective total attendance of 300, 603, 620, 844, 1,529, 1,632, and 1,732, respectively. Likewise, attendance figures bottomed out seven times; namely, Columbus, Ohio, 1918; Davis, California, 1931; Lincoln, Nebraska, 1937; Moscow, Idaho-Pullman, Washington, 1939; Columbia, Missouri, 1943; Davis, California, 1952; and Stillwater, Oklahoma, 1957. The respective total attendance at these meetings was 48, 150, 411, 390, 318, 640, and 1,187. However, with two exceptions, each low in attendance was followed by a succeeding higher low. These lows could be accounted for because of the 1918 flu epidemic, geographic location, and gas-rationing. Actually, the lowest attendance at any regularly scheduled

TABLE 1

Attendance at the annual meetings of the American Dairy Science Association, 1906 to 1958, inclusive

Meeting	Location	Year	Attendance				Representation	
			Men	Women	Children	Total	States[c]	Countries[f]
1st	Urbana, Illinois	1906	18			18	7	1
2nd	Chicago, Illinois	1907	21			21	....	..
3rd	Ithaca, New York	1908	47			47	18	2
		1909-1916	(No attendance record)				....	..
12th	Columbus, Ohio	1917	70			70	27	..
13th	Columbus, Ohio	1918	48	(Flu epidemic)		48	....	..
		1919-1921	(No attendance record)				....	..
17th	St. Paul, Minnesota	1922	300[a]				....	..
22nd	East Lansing, Michigan	1923-1926	(No attendance record)					
		1927	68	(In picture taken)			....	..
		1928-1929	(No attendance record)				....	..
25th	Ames, Iowa	1930	.....			250[b]	....	..
26th	Berkeley-Davis, California	1931	.....			150[b]	....	..
27th	Lexington, Kentucky	1932	200[b]	50[b]		280[b]	....	..
28th	Urbana, Illinois	1933				.....	....	..
29th	Ithaca-Geneva, New York	1934	250[b]	100[b]		350[b]	....	..
30th	St. Paul, Minnesota	1935	325	121	33	479	37	2
31st	State College, Pennsylvania	1936	355	134	144	603	35	2
32nd	Lincoln, Nebraska	1937	303	76	32	411	40	6
33rd	Columbus, Ohio	1938	447	125	48	620	40	3
34th	Moscow-Pullman, Washington	1939	196	113	81	390	34	2
35th	W. Lafayette, Indiana	1940	480	235[a]	.....	715	....	..
36th	Burlington, Vermont	1941	492	352[a]	.....	844	....	..
37th	East Lansing, Michigan	1942	394	93	79	566	39	1
38th	Columbia, Missouri	1943	.....	.....	.....	318	34	1
39th	Columbus, Ohio	1944	.....	.....	.....	384	38	1
40th	Chicago, Illinois	1945	(Officers and two guests only)					
41st	Ames, Iowa	1946	532	225[c]	.....	757	43	5
42nd	Guelph, Ontario	1947	583	328[c]	.....	911	43	9
43rd	Athens, Georgia	1948	600	356[c]	.....	956	44	1
44th	St. Paul, Minnesota	1949	780	262	73	1,115	46	9
45th	Ithaca, New York	1950	940	394	195	1,529	47	8
46th	Knoxville, Tennessee	1951	701	159	110	970	43	9
47th	Davis, California	1952	371	167	102	640	43	6
48th	Madison, Wisconsin	1953	891	296	219	1,632[d]	46	13
49th	State College, Pennsylvania	1954	875	321	232	1,428	46	6
50th	East Lansing, Michigan	1955	842	316	202	1,360	49	9
51st	Storrs, Connecticut	1956	871	318	226	1,415	45	10
52nd	Stillwater, Oklahoma	1957	746	227	214	1,187	47	6
53rd	Raleigh, North Carolina	1958	985	364	342	1,732[g]	46	9

[a] Estimated attendance at banquet.
[b] Approximate number.
[c] Women and children.
[d] Including 100 guests and 126 affiliates.
[e] Including District of Columbia.
[f] Including Alaska and Hawaii.
[g] Including guests.

meeting during the past thirty years was in Chicago, in 1945, when officers and necessary guests convened to conduct the annual affairs of the society.

Has Yearly Attendance Kept Pace with Membership?

The relationship between annual attendance and membership is shown also in Figure 1. Generally, a parallel relationship exists between the two. None the less, yearly membership usually far exceeds the total attendance at the meeting. Not since 1927 has attendance at the annual meeting exceeded membership for the same year. Geographic location of the meetings does affect the number in attendance, but seems to have had little influence on the total membership.

Attendance Records

Several meetings stand out for certain records. For example,

a) North Carolina has the record for the greatest total attendance—1,732 in 1958, exceeding Wisconsin's 1953 record by 100.

b) North Carolina also holds the record attendance for men—985 in 1958, beating the previous record held by Cornell of 940 in 1950.

c) Michigan, 1955, is the only meeting from which representatives came from all 48 states and the District of Columbia. (Attendance from the then Alaska Territory was placed under another classification.)

d) North Carolina had the greatest children enrollment, 342 in 1958, beating out Pennsylvania State University's previous record of 232 in 1954.

e) Cornell holds the record for the largest registration of women, 394 in 1950, exceeding Georgia's previous record of 356 in 1948 by 38, and North Carolina's 1958 attendance of 364 by 30.

f) North Carolina hosted the highest number of women and children combined, 706 in 1958.

g) Wisconsin is first in having the greatest number of foreign countries represented, 13 in 1953.

h) Since World War II, Tennessee is first in the percentage of men in total attendance, 72.7%. Wisconsin, 1953, and North Carolina, 1958, had the lowest percentages of men in attendance, with 54.6 and 56.9%, respectively.

i) From 1949 to 1958 inclusive, 10 years, men, women, and children averaged 62.8, 22.2, and 15.0% of the total attendance.

j) Prior to World War II, the University of Vermont, Burlington, 1941, had the highest total attendance ever recorded at the 36 meetings held to that time. Since World War II, this record has been broken 11 times, by meetings at Guelph, Athens, St. Paul, Ithaca, Knoxville, Madison, State College, East Lansing, Storrs, Stillwater, and Raleigh.

k) The average total attendance of the 13 meetings since World War II has been 1,202, consisting of an average of 747 men and 434 women and children.

These figures might well serve as a general guide to those planning accommodations at the annual meetings. Due recognition must be given for the natural effects of geographical location upon attendance, bearing in mind that the center of membership lies east of the Mississippi and north of the Mason-Dixon line.

ABSTRACTS OF PAPERS TO BE PRESENTED AT THE FIFTY-FOURTH ANNUAL MEETING of the AMERICAN DAIRY SCIENCE ASSOCIATION

University of Illinois
Urbana
June 15–17, 1959

MANUFACTURING SECTION

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M1. Preparation of calcium-sensitive α-casein. C. A. ZITTLE, Eastern Utilization Research and Development Division, USDA, Philadelphia 18, Pennsylvania.

The term calcium-sensitive α-casein is used for that fraction of α-casein which is precipitated with low concentrations of $CaCl_2$. When the concentration of the sensitive α-casein is 1% and the pH 6.8, it is almost completely precipitated by 10 m*M* $CaCl_2$ per liter, a concentration that will also precipitate paracasein completely. The calcium-sensitive α-casein can be prepared from acid-precipitated whole casein by a simple modification of the procedure for preparing α-casein by differential solubility in urea solutions. The whole casein is dissolved in 6.6 *M* urea and α-casein is precipitated from β- and γ-caseins by dilution to 4.6 *M* urea. The α-casein is redissolved in 6.6 *M* urea and for complete solution NaCl is added. In the usual procedure, 1.59 g. is added per 150 ml.; if twice as much NaCl is used, in the subsequent precipitation by dilution with an equal volume of the water, the stabilizing fraction of α-casein will remain in solution and only the calcium-sensitive α-casein will precipitate. The yield is about 30% of the total casein. Some of the calcium-sensitive α-casein is still present in the stabilizing fraction, as well as some β-casein.

M2. Effect of homogenization upon the fat-protein interaction in milk. K. K. FOX, JEAN E. CAHA, VIRGINIA H. HOLSINGER, AND M. J. PALLANSCH, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

A sedimenting fat-protein complex is produced in milk and concentrated milk products by homogenization. The per cent of fat which interacts with protein to form a sedimentable complex depends upon the concentration of each and upon the homogenization pressure. For example, at 2,000 p.s.i. 17% of the fat in milk of 12.4% total solids (T.S.) and 3.3% fat interacts with protein; at 8,000 p.s.i., 35–40% reacts. When the same milk was concentrated to 50% T.S., the comparable values were 20 and 75%.

Experiments to determine the manner in which the fat-protein complex is formed indicate that the conditions existing at the homogenizer valve are necessary for complex formation. The principal protein, if not the only one, involved in the interaction is the caseinate micelle. Experiments show that the larger the micelle, the more fat is complexed at a given homogenization pressure.

M3. Characteristics of the adsorbed proteins of the homogenized milk fat-globule. R. H. JACKSON AND J. R. BRUNNER, Michigan Agricultural Experiment Station, East Lansing.

The combined effects of homogenization and heat treatment on the proteins of the fat membrane were studied. The adsorbed proteins were isolated from the processed milk by churning five-times-washed cream. Pasteurization treatments were varied and included HTST processing at 162° F. for 16 sec. to batch processing at 175° F. for 20 min.

Yields of protein material from each of the processed milks indicated that the heat treatment played an important role in the amount and nature of the proteins adsorbed on the fat surfaces. Electrophoretic studies showed that the native membrane-protein complex was not removed by homogenization, but that casein and whey proteins were adsorbed. Interestingly, the casein isolated from the adsorbed protein mixture always contained another component, with a mobility between that of α- and β-casein. This component was precipitated at pH 4.6, was heat-stable at 90° C., and had a large amount of lipid material associated with it.

M4. Changes in milk proteins accompanying the growth of psychrophilic bacteria as determined by paper electrophoresis. J. D. SKEAN AND W. W. OVERCAST, University of Tennessee, Knoxville.

In high-quality pasteurized skimmilk inoculated with pure-culture psychrophilic bacteria and subsequently stored at 4± 1° C., bacterial growth and protein changes were followed by agar plate counts and paper electrophoresis, respectively. Comparative total and psychrophilic bacteria counts, along with electropho-

retic analyses, were made at 14-day storage intervals. In preparation for electrophoresis, the casein and whey were separated by precipitation of the casein at pH 4.6 with lactic acid, followed by filtration. After thorough washings in distilled water at 4–6° C., the casein was lyophilized to dryness. The whey was passed through a Seitz filter, dialyzed against distilled water at 4–6° C. for 44 hr., and then lyophilized to dryness. Casein and whey protein preparations adjusted to 1% protein nitrogen content (micro-Kjeldahl method) in barbital–sodium barbital buffers (pH 8.6) were electrophoretically analyzed, using a Spinco Model R Paper Electrophoresis System. The electrophoretic patterns obtained indicate that changes occurring in the proteins of skimmilk supporting the growth of these bacteria involve: (1) the total protein content, (2) the relative percentages of components present, and (3) the appearance of new components.

M5. Protein complex formation in evaporated milk. J. C. TRAUTMAN AND A. M. SWANSON, University of Wisconsin, Madison.

Previously reported electrophoretic evidence has suggested that forewarming heat treatments induce a stable complex between the α-casein components and β-lactoglobulin, and that this complex could be prevented by sulfhydryl group blocking agents. This evidence suggested the addition of the blocking agent to evaporated milk. During the manufacture of evaporated milk on a laboratory scale, samples were made 10^{-4} M with p-chloromecuribenzoate before and after forewarming. The samples containing the blocking agent before forewarming were unstable to the sterilizing temperatures, while the sample to which the blocking agent was added after forewarming appeared identical to the normal evaporated milk samples.

This evidence appears to relate protein complex formation as reported from electrophoretic studies to heat stability in evaporated milk. The complex appears to be necessary to obtain heat stability to sterilizing temperatures. In addition, this complex formation was found to occur during forewarming treatments of the whole milk and not during sterilization treatments of the concentrate. This points out the possibility of considering the proteins of forwarmed milk as a "new" protein system.

M6. Characterization of milk serum protein component 5. ROBERT JENNESS, University of Minnesota, St. Paul.

The protein component of acid milk serum having an electrophoretic mobility of pH 8.6 intermediate between those of α-lactalbumin and β-lactoglobulin is designated here as Component 5. This component was precipitated along with casein, lactoperoxidase, and other miscellaneous proteins by saturating raw skimmilk with NaCl. The precipitate was redispersed and casein removed at pH 4.6. After concentrating the filtrate, lactoperoxidase was adsorbed on an ion exchange resin, and Component 5 was selectively precipitated by careful adjustment of the pH in the neighborhood of pH 4.5. The most highly purified concentrates that were prepared contained over 90% of material with the mobility of Component 5 at pH 8.6, but were somewhat more heterogeneous at pH 2.3. The electrophoretic mobility at 0.1 ionic strength is -4.5×10^{-5} cm.^{2} V.$^{-1}$ sec.$^{-1}$ at pH 8.6 in veronal and $+3.6 \times 10^{-5}$ cm.^{2} V.$^{-1}$ sec.$^{-1}$ at pH 2.3 in HCl–NaCl. Preliminary data indicate that Component 5 contains 1.2–1.3% phosphorus, is soluble in $CaCl_2$ at 0.02 M and 0.2 M concentrations, and is not clotted by rennin, either in the presence or in the absence of calcium (.033 M). It differs from any of the fractions that have been reported to have been isolated from α-casein. Some evidence has been secured that Component 5 is the heat-labile loaf volume depressant of raw milk. The proteins of acid and rennet whey, which contain Component 5, exert a pronounced depression of loaf volume when incorporated into dough at the concentration of 0.5 g. of whey protein per 100 g. of flour. The proteins of NaCl serum which does not contain this component lack the effect.

M7. Effect of steam injection and vacuum treatment on the flavor of milk. W. F. SHIPE, Cornell University, Ithaca, New York.

For this study, a 4,000-lb. per hour, double-chamber Cherry-Burrell Aro-Vac unit was used in conjunction with a HTST pasteurizer. The flow sequence was as follows: Pasteurizer to Aro-Vac unit to homogenizer to regenerator to surge tank to bottle filler. Samples of pasteurized milk (A), Aro-Vac-treated milk (B), and bottled milk (C) were collected twice a week for 6 wk. Six experienced judges graded the samples when fresh and after seven and 14 days of storage at 40° F. The average flavor scores of the fresh products were 38.3, 38.9, and 38.9 for Samples A, B, and C, respectively. The corresponding scores were 37.0, 38.3, and 38.0 after seven days and 36.0, 38.0, and 36.1 after 14 days. Although the differences in scores were small, they are considered significant because the judges consistently placed the samples in the same order.

The Aro-Vac treatment reduced the "feedy" flavor and prevented oxidation. The deterioration of the pasteurized samples was due primarily to oxidation, whereas bacterial action was the primary cause in the bottled samples.

M8. Effect of steam injection and vacuum treatment on bacterial quality of milk stored for seven and 14 days at 40 and 50° F. G. V. GUDNASON, W. F. SHIPE, AND R. F. HOLLAND, Cornell University, Ithaca, New York.

For this study, a 4,000-lb. per hour, double-chamber Cherry-Burrell Aro-Vac unit was used in conjunction with an HTST pasteurizer. Samples of pasteurized milk (A), Aro-Vac-treated milk (B), and bottled product (C) were col-

lected twice a week for 7 wk. (The flow sequence in this milk system was as follows: Pasteurizer to Aro-Vac to homogenizer to regenerator to surge tank to bottle filler.) Standard plate counts (SPC) and psychrophilic counts (PsC) were made from these samples according to Standard Methods, using incubation temperatures of 32 and 5° C., respectively. Milk held at 40° F. for seven and 14 days showed only slight increases in the SPC and PsC counts of A and B. Samples C, however, increased both in SPC and PsC from 210 and 0, respectively, to 150,000 and 340,000 in 14 days. The SPC for A and B, held at 50° F. for 14 days, were 10,000,000 and 40,000,000, respectively. There was no increase in the PsC of these samples. The SPC for Samples C were 8,000,000 and 260,000,000 after seven and 14 days, respectively. The corresponding PsC were 6,000,000 and 26,000,000.

M9. Removal of feed flavors from milk by vacuum pasteurization. J. D. Wynn and J. R. Brunner, Michigan Agricultural Experiment Station, East Lansing.

The volatile flavor components of mixed-herd milk from cows on Michigan summer pasture were collected before and following a steam-injection, vacuum-pasteurization treatment. Similar collections were made from the vapor passing through the vacuum chamber during the processing of the milk. Only the volatiles which were condensed in the liquid-air traps of a low-pressure collection system were studied. The condensed volatiles were resolved by gas-liquid phase chomatography.

Approximately 95% of the volatile components were removed from the milg by the vacuum-pasteurization treatment, employing a maximum temperature of 180° F. and flashing to approximately 164° F. (18 in. of vacuum). Acetaldehyde, acetone, methyl sulfide, and an unidentified aldehyde or ketone, in increasing order of concentration, were recovered from the expelled vapors. These same components were absent or present only in trace quantities in the processed milk. Two components of higher molecular weight and in relatively low concentrations were not removed from the milk processed in this manner. Raw milk which was scored at 37 and criticized as "feedy" in flavor was scored at 39 following processing.

Vacuum pasteurization, as applied in this study, was successful in removing the "feedy" or "cowy" flavor components from summer milk.

M10. Consumer acceptance of milk beverages as affected by fat and solids-not-fat content.* J. W. Stull and J. S. Hillman, University of Arizona, Tucson.

A consumer-preference study was conducted to provide statistically reliable data on which standards and practices relating to the optimum composition of milk beverages can be based.

The addition of solids-not-fat (SNF) to milk beverages is not provided for in most standards. There is a general awareness, however, that the addition of from 0.5 to 2.0% SNF to either whole, low-fat, or nonfat milk beverages improves palatability, flavor, food value, and utilization.

Taste discrimination or threshold tests involving 2,132 paired and triangulation decisions were conducted with adult men and women who were not trained dairy products judges. The results showed that people could consistently differentiate between milk beverages with variations in fat and SNF contents of 0.5 and 1.0%, respectively.

Paired preference comparisons within and between each beverage class (whole, low-fat, and nonfat beverages) were made with approximately 10,000 persons in retail food markets, public gatherings, and public schools. Beverages with 0.5 to 1.0% added SNF were preferred. There was no significant difference in preference for a regular whole milk (3.5% fat, 8.5% SNF) when compared with a low-fat beverage (2.0% fat, 10% SNF).

M11. Effect of the development of oxidized flavor on the polyunsaturated fatty acids of milk lipids. L. M. Smith and W. L. Dunkley, University of California, Davis.

In a general study of effects of oxidation on composition of milk lipids, the amounts of polyunsaturated acids in the triglyceride and phospholipid fractions of milk isolated before and after development of oxidized flavor were compared. Pasteurized milk (10% fat) that was susceptible to oxidized flavor was divided into two lots. The nonoxidized control was examined immediately and the oxidized lot 48 hr. later (after adding 0.1 p.p.m. of copper and holding at 5° C.). Most of the triglycerides were recovered from each lot by churning the milk, melting the butter granules, and filtering the clear fat. The butter serum and buttermilk were combined and extracted with ethanol, ether, and pentane. The lipids were recovered and the remainder of the triglycerides were separated from the phospholipids by chromatography. In four experiments, differences in the iodine values and polyunsaturated acids from the normal and distinctly oxidized milks were small, but were greatest in the phospholipid fractions. Analytical data for the phospholipid fraction from the normal sample of one experiment (with data for the oxidized sample in parentheses) were: iodine value, 47.59 (45.20); % phosphorus, 3.50 (3.40), and % nitrogen, 2.76 (2.74). Corresponding percentages of fatty acids were: conjugated dienoic, 0.6 (0.5); monoethenoid, 26.4 (26.1); linoleic, 4.5 (4.0); linolenic, 1.7 (1.7); arachidonic, 1.3 (1.3), and pentaenoic, 1.3 (1.1).

* This research was supported by a grant from the American Dairy Association, Chicago, Illinois.

M12. Role of a chelating compound in the inhibition of oxidized flavor. R. L. KING AND W. L. DUNKLEY, University of California, Davis.

Addition of 2 g. EDTA (ethylenediaminetetraacetic acid) per liter of milk effectively inhibited the development of spontaneous and copper-induced oxidized flavor (0.1 p.p.m. added copper). Addition of EDTA also resulted in changes in the rate of oxidation of ascorbic acid and the state and distribution of copper in milk. In samples not containing added copper, the rate of oxidation of ascorbic acid was increased when the level of EDTA was increased. In samples containing 0.1 p.p.m. added copper, the rate of oxidation of ascorbic acid was markedly decreased by the initial level of EDTA, but this rate was increased slightly by further addition of EDTA. In milk labeled with Cu^{64} in natural association, the addition of 1 g. EDTA per liter resulted in chelation of 37% of the copper in skimmilk and 50% from washed fat globules as determined by dialysis for 24 hr. at 3° C. In milk containing Cu^{64} by direct addition (0.2 p.p.m. total copper), the same level of EDTA chelated over 50% of the added copper from skimmilk and 100% from washed fat globules. The inhibition of oxidized flavor by EDTA is considered to be the result of removing catalytic copper from a key site in the fat globule membrane.

M13. Dihydroquercetin as an antioxidant for milk. G. A. RICHARDSON AND D. R. ERICKSON, Oregon State College, Corvallis.

Although previous studies showed that several flavone-type compounds are effective antioxidants for milk fat and for milk, their use in milk is, in general, limited by low water solubility and the tendency to confer foreign flavors. The water solubility (0.5%), lack of color, the absence of undesirable flavor in effective concentration, and the nontoxicity of dihydroquercetin prompted this study.

Dihydroquercetin (DHQ) at the 1 mg. % level, added before or after pasteurization, was found to prevent oxidative flavor changes in susceptible mixed milk of two cows, in the presence of 1 p.p.m. of copper added after pasteurization, for at least three days in dark storage at 1–2° C. No foreign flavor was detected. Quercetin (Q) was effective at the same concentration, but existed as a dispersion yielding a yellowish sediment on standing.

Oxidative changes were determined organoleptically and by the 2-thiobarbituric acid test. Oxidation-reduction potential values increased progressively during storage in all milks containing added copper; those containing DHQ or Q, especially when added prior to pasteurization, remained poised at higher Eh values than those with copper alone. The anomaly of antioxidant activity in the face of increased Eh suggests a synergism involving ascorbic acid and/or tocopherol(s).

M14. Removal of Sr^{89} and Ca^{45} from milk with ion exchange resins. D. G. EASTERLY, B. J. DEMOTT, AND R. G. CRAGLE, University of Tennessee, Knoxville, and UT-AEC Agricultural Research Laboratory, Oak Ridge, Tennessee.

Milk from cows administered single oral quantities of Sr^{89} and Ca^{45} was mechanically separated into cream and skimmilk. One liter of the skimmilk was passed through a column containing 50 g. of a resin. Resins used were Dowex 50 W, 50–100 mesh, with a cross-linkage of 4%, Dowex 50 w, 50–100 mesh, with a cross-linkage of 12%, and Duolite C-20.

The Ca form of each resin was more effective in removal of the isotopes than the Na form, The data obtained indicate that, in the second 100 ml. passed through the column, between 70 and 80% of the Sr^{89} and Ca^{45} in the milk was removed and in the tenth 100 ml. 50% of each isotope was removed using either of the Dowex resins. The Duolite was not as efficient, when used under these conditions.

M15. A partial differentiation of the esterases of milk. T. L. FORSTER, M. W. MONTGOMERY, AND H. A. BENDIXEN, Washington Agricultural Experiment Station, Pullman.

Organo-phosphorus inhibitors (e.g., Isopestox, Parathion, DFP, TEPP) have been extensively used in differentiating the esterases of biological fluids and tissues. The influence of various concentrations of these inhibitors on the activity of the enzymes of milk toward phenyl propionate, phenyl acetate, tributyrin, benzyl butyrate, and the butyryl and stearoyl esters of 2-naphthol-6-So_3Na were studied, using Warburg manometric techniques at pH 8.0 and 37° C. Raw, mixed vat milk served as the enzyme source. Three distinct esterases or esterase systems were evident. One was responsible for about 95% of the activity of the milk enzymes toward tributyrin, benzyl butyrate, and the butyryl and stearoyl esters of 2-naphthol-6-SO_3-Na, the second for 60–70% of their activity toward phenyl propionate, and the third for about 90% of their activity toward phenyl acetate. Each of these esterases appeared able to hydrolyze one or more of the other substrates to a limited extent. The activity of these three enzymes accounted for essentially all of the observed hydrolysis of the substrates studied by the enzymes of the milk samples used. At least two, and perhaps all three, of these esterases may be involved in the hydrolysis of milk fat and the subsequent development of rancidity.

M16. Some observations on lipase activity. B. L. HERRINGTON AND E. S. GUTHRIE, Cornell University, Ithaca, New York.

To learn more about the disputed relationship between stage of lactation and the incidence of rancidity in milk, it was planned to measure the acid degree of the fat in the milk of 20 individual cows at intervals during their

entire lactation periods. Measurements were made on the milk when fresh and after 24 hr. at 40° F. Eight hundred analyses were made; 16 cows completed the experiment. Acid degree values in the last month of lactation, even in stripper milk taken 48 hr. after the last regular milking, were not unusually high. The maximum values occurred most frequently (five cows) in the first month of lactation. However, those cows yielding the most active milk had the longest lactation periods and were milked in the 13th and 14th mo.

M17. Effect of penicillin and aureomycin upon the phosphatase enzyme system of milk.* C. V. MORR, The Ohio State University, Columbus.

The effect of penicillin (0-25 U/ml) and aureomycin (0-25 μg/ml) on the activity of milk phosphatase was determined by a modified Scharer laboratory method.

A total of 13 assays were conducted on six samples of raw milk containing no antibiotic. By measurement of the velocity of enzyme reaction at different substrate concentrations, and by application of conventional equations, an average value for the Michaelis constant was found to be 25.25×10^{-4} M per liter, with a rather large amount of variation among different milks.

Application of this technique to the same milks inoculated with penicillin revealed an average enzyme inhibition of 22.4% in the presence of relatively low substrate concentrations (0.004 molar disodium phenyl phosphate). However, this inhibition was reduced to zero with higher substrate concentrations (0.02 molar). The amount of inhibition observed would not be sufficient to alter the reliability of the phosphatase pasteurization test when applied to heated milk containing much smaller quantities of both enzyme and antibiotic.

Aureomycin trials produced results of a variable nature and no relationship between antibiotic level and enzyme activity could be determined.

Other trials conducted showed that neither penicillin nor aureomycin caused an alteration in the prevalance or degree of enzyme reactivation in milk heated to 210° F., for 10 sec. or 145° F., for 30 min.

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*Supported by a grant from the U. S. Public Health Service through the Ohio State University Research Foundation.

M18. Differences in the viscosity and voluminosity of centrifuge sediments from milk due to suspending medium and to sediment fraction. C. H. WHITNAH AND R. BASSETTE, Kansas State College, Manhattan.

Four similar sediments were isolated from a sample of skimmilk with a Sharples supercentrifuge. They were resuspended, respectively, in water, a salt solution, rennet whey, and supercentrifuge whey (after long high-speed centrifuging). The four suspensions all differed in effects of temperature on viscosity. High-speed centrifuge whey seemed more like undisturbed milk serum and was, therefore, selected as the medium for resuspending four fractions of sediments from several later milks.

Sediments One and Two were scraped from the bottom and top thirds of the bowl after low-speed centrifuging. Sediments Three and Four were similar fractions after high-speed centrifuging of liquid from the low-speed centrifuging. The four fractions differed in ratio of viscosity to concentrations and in voluminosity.

M19. A quantitative test for carrageenin ester sulphate in milk. P. M. T. HANSEN AND R. MCL. WHITNEY, University of Illinois, Urbana.

Carrageenin has found wide use in the dairy industry as a stabilizer in various products. Therefore, a quantitative test for its presence in milk is desirable and for this purpose advantage may be taken of its high ester sulphate content. As developed, the test consists of: (a) dialysis of the sample to remove inorganic sulphate, (b) hydrolysis in hydrochloric acid to liberate the ester sulphate, (c) treatment with charcoal and filter-aid followed by filtration to clarify the samples, (d) gravimetric determination of sulphate as barium sulphate in the filtrate, and (e) calculation of the ester sulphate content as the difference between a sample and a control containing the reagents used.

The effects of the following variables upon the determination were investigated: hydrolysis time, strength of acid, amounts of charcoal and filter-aid, type of charcoal, dilution of samples, and dialysis. The recoveries in milk ranged from 93.0 $\pm$ 4.9% to 96.9 $\pm$ 3.2% for levels of carrageenin concentrations at 0.05 and 0.10%, respectively.

The test has been applied to various salts of λ- and κ-carrageenin and, within the two fractions, the results yielded a constant ester sulphate content for the carrageenin anion. These values were supported in part by structural considerations.

M20. Chromatographic determination of sugars in ice cream. T. C. CHOU AND JOSEPH TOBIAS, University of Illinois, Urbana.

Sugars in ice cream were separated by descending chromatography. A diluted sample of melted ice cream was applied directly on sheets of Whatman No. 1 chromatographic paper. The irrigation solvent consisted of two parts of *n*-butanol, one part ethanol, and one part water. The sugars which were thus separated were, in the order of decreasing Rg values: fructose, dextrose, sucrose, maltose, lactose, isomaltose, higher sugars, and dextrins. Higher sugars and dextrins were not present in sufficiently high concentrations in ice cream to produce visible spots upon development. Guide strips

were developed by spraying with aniline phthalate or orthoaminobiphenyl phosphate reagents. The corresponding areas on the undeveloped strips were cut out and the sugars were extracted with water, using filter tubes packed with glass wool. Quantitative determinations of the sugars were performed on an aliquot of the water extract by a colorimetric procedure with anthrone. Data are presented in support of the validity of the various steps involved in this procedure.

M21. Specifications for the spreadability of butter. R. R. RIEL, Dairy Technology Research Institute, Ottawa, Canada.

The spreadability of butter is defined in objective values obtained with the spreadability instrument developed by Huebner and Thomsen (University of Wisconsin). Butter samples and the instrument were placed in a room where the temperature was controlled. The spreadability of the butter was varied by changing the temperature of the room.

Judgments obtained to date from an organoleptic panel of 15 people indicate that desirable spreadability of butter ranged between 150 and 400 g. of resistance, whereas acceptable spreadability of butter ranged between 100 and 800 g.

For most North American consumers, it would be desirable that the spreadability of butter be at least acceptable from the temperature of the home refrigerator (40–45° F.) to the most usual room temperatures (70–75° F.).

M22. The hardness and spreadability of butter as affected by edible additives.* J. J. BETSCHER, T. KRISTOFFERSEN, AND I. A. GOULD, The Ohio Agricultural Experiment Station, Wooster.

The possibility of improving the spreading characteristics of butter through the addition of various edible substances (28) was investigated.

The consistency of the butters was evaluated, using a specially constructed "consistency meter". The experimental procedure yielded variations up to 10% among replicate trials and, therefore, only those compounds which produced an effect exceeding this level were considered beneficial. The hardness and spreadability of the control samples ranged from 80 to 180 g. and from 368 to 790 g., respectively.

Improvement obtained in hardness (h) and spreadability (s), by use of various effective compounds in cream at the 2–3% levels on the fat basis, is as follows: Myvacet Type 9-40 (distilled monoglycerides), $h = 19\%, s = 12\%$ vegetable lecithin, $h = 0\%$, $s = 18\%$; Mulcrest (mixture of mono-, di-, and triglycerides and vegetable gum in a water solution), $h = 11\%, s = 14\%$; and Atmos 300 (mono- and diglycerides in propylene glycol), $h = 16\%, s = 17\%$. Appreciable improvement was noted when the following materials at the 2–3% level were added to butter prior to working: Tween 40 (polyoxyethylene sorbitan monopalmitate), $h = 33\%, s = 24\%$; Span 80 (sorbitan mono-oleate), $h = 15\%, s = 19\%$; Atmos 300, $h = 29\%, s = 21\%$; skimmilk solids, $h = 4\%, s = 15\%$; buttermilk solids, $h = 14\%, s = 13\%$, and vegetable lecithin, $h = 28\%, s = 15\%$.

Considerable variation in results occurred among several trials using the same additive, indicating the presence of unexplained factors which prevent the achievement of uniformity in the body of butter by the use of additives alone.

M23. A solids-not-fat test for milk using density plastic beads as hydrometers. N. S. GOLDING, The State College of Washington, Pullman.

Realizing that existing solids-not-fat (S.N.F.) tests for milk were not generally acceptable to the dairy industry, research work was started 6 yr. ago to obtain the following objectives: Accuracy, speed, low cost, simplicity, and small milk sample. A search was made for a suitable plastic to replace glass spheres often used to determine density, physical properties, filling and fabrication of the selected plastic, and the application of two types of plastic hydrometers for determining S.N.F. in the milk from individual cows of three breeds.

The apparatus consists of a 4-oz. sample bottle containing ten different density plastic beads, held in by a snap-in plastic grid. The number of beads sinking at a given temperature determine the density of the milk, and a check can be made by determining the number that float. This paper includes: designation of each gravity sphere by a different color, procedures to avoid any possible error in reading, pilot plant manufacture and improvement, further use of the test, and obtaining acceptance of the sphere test.

M24. Tests for evaluating the milk plasma phase of whole milk. W. A. KRIENKE, Florida Agricultural Experiment Station, Gainesville.

From standpoints of milk pricing, nutritional value, product yield, and controlled properties of manufactured products there is need for means of evaluating the plasma phase of whole milk. Currently, considerable interest is being given to solids-not-fat (S.N.F.) by formula based on fat and lactometer. Data on studies here show such S.N.F. values to be inadequate and misleading. When supplemented by values for protein (formal titration), titra-

* A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service.

table acidity, pH, and chlorides, a meaningful evaluation of milk plasma is possible.

This study was conducted on daily milk samples as follows: 120 individual cows—six consecutive days; 11 individual cows—75 consecutive days; mixed milk of about 100-cow herd—irregular intervals through 150 days. Some samples of whole milk were practically identical in calculated S.N.F. values, but differed considerably in protein content; whereas, others of identical or nearly the same protein content differed in calculated S.N.F. values. By applying established scientific explanations based on chloride values, together with pH and/or titratable acidity values, certain complementary relationships are shown. Such data may be useful also in breeding programs aimed at increasing the protein content of milk.

M25. An improved procedure for the determination of milk proteins by dye binding. U. S. Ashworth, State College of Washington, Pullman.

The method of Udy has been improved by applying the principle of relative absorbance described by Hiskey *et al.* (*Anal. Chem.*, **22**: 1464. 1950). By using a short light path cell (0.75 mm.) and setting the Model B Beckman spectrophotometer at zero, with a dye solution containing 0.32 mg. of Orange G per ml., maximum sensitivity can be achieved. At a wave length of 0.475 μ, there is a linear relation between absorbance of the unreacted dye and the amount of protein in the sample which should range between 25 and 90 mg. Twenty-five milliliters of a 0.100% Orange G dye solution is the only reagent needed.

The dye-binding capacity of milk proteins is not constant, which necessitates the use of a standard curve prepared from known milk samples. Under carefully controlled conditions, the results have about the same precision as macro-Kjeldahl determinations. The minimum of 0.32 mg. per milliliter for unreacted dye insures that sufficient dye has been present to react with all of the protein.

The dye-binding procedure is particularly adaptable to routine work. No more time is required per sample than is required for the Babcock test.

M26. A direct titrimetric method employing a silver electrode for estimating the chloride content of cow's milk. D. H. Kleyn and B. L. Herrington, Cornell University, Ithaca, New York.

A quick and accurate method has been developed in which wet or dry ashing is avoided. The method has been found to compare favorably with the thiocyanate titration method; and duplicates are found to agree within one-tenth of a milliliter which is equivalent to 0.002% chloride.

An ordinary Beckman pH meter is employed by substituting a silver electrode for the glass electrode. A silver nitrate solution of such strength so that each milliliter is equivalent to 0.02% chloride is added from a burette, until a predetermined E.M.F. is reached. The chloride content is then easily calculated by multiplying the number of milliliters used by 0.02. Errors due to proteins and phosphates are eliminated by conducting the titration in an acidic medium. Comparative results are as follows:

Com.	Titrimetric Method			Thiocyanate Method		
No.	*(A)*	*(B)*	*Av.*	*(A)*	*(B)*	*Av.*
	(% chloride)			*(% chloride)*		
1	0.102	0.102	0.102	0.099	0.104	0.102
2	0.081	0.083	0.082	0.078	0.080	0.079
3	0.081	0.081	0.081	0.081	0.083	0.082
4	0.084	0.085	0.085	0.081	0.082	0.082

M27. Photometric determination of fat in milk. G. Haugaard and J. D. Pettinati, National Dairy Products Corporation, Oakdale, Long Island, New York.

The theoretical considerations and practical application of a photometric procedure for milkfat determination of homogenized milk are presented. Conditions are given which reduce the factors that influence the light scattering of milk to two. Dilution of samples eliminates the influence of globule-size distribution as a variable. A chelating agent in the diluting solution destroys the micelles to eliminate casein turbidity. Using a favorable wavelength of light, in the visible region, only two independent measurements are necessary to determine either or both of the remaining two variables; namely, average globule size and fat content. The globule size range of homogenized milk is sufficiently narrow so that the mathematical treatment is simple. Two equations are derived, which can be used to construct a nomogram.

The total transmitted light through a thin layer of the diluted sample and the parallel transmitted light constitute two independent measurements. From readings of these two quantities on known samples, a nomogram was constructed from which fat content and homogenization degree of unknown samples were then read. The method was tested by analysis of a large number of commercial milk samples, and the results agreed well with the Babcock values. A bench homogenizer is used to prepare small samples of homogenized milk for determination. Applicability to other dairy products is discussed.

M28. Photometric fat test for ice cream mix. J. D. Pettinati and G. Haugaard, National Dairy Products Corporation, Oakdale, Long Island, New York.

A photometric fat method developed for whole milk was successfully applied to the determination of fat in ice cream mix. Two independent optical densities are measured, to

determine fat content and degree of homogenization simultaneously. The procedure requires dilution of samples to a concentration of about 1% fat. To an accurately weighed 1.5-g. portion of mix are added 20 cc. of a Versene diluting solution. As with whole milk, a 500-μ light-path cell and light of 0.6 μ wavelength are used. Samples tested were obtained from plant production batches. Data obtained from comparative tests with the Mojonnier method are shown graphically. The accuracy and precision of the method compare favorably with those of the Mojonnier. Being an instrumental method, there is a minimum of opportunity for personal error. No exceptional skill or practice is required to make the dilutions, measure on the instrument, and read the nomogram. The time required to make an individual test is from 3.5 to 5 min., as compared to 40 min. by Mojonnier.

M29. Oxidation-reduction measurements on Cheddar cheese.* T. KRISTOFFERSEN AND I. A. GOULD, The Ohio State University, Columbus.

Changes in oxidation-reduction potential (Eh) during cheese ripening were followed by (a) the use of dyes (Janus green, E'o = −0.261, pH 7, 2nd stage, or Neutral red, E'o = −0.324, pH 7), and (b) the imbedding of platinum electrodes in the curd at hooping.

The "dye cheese" (12 lots) had pH's of 5.1 to 5.6 after from two to three days. For cheese in the pH 5.1 to 5.3 range, the color was relatively uniform and the initial Eh approximated −270 mV. The Eh of this cheese increased to about −225 mV and −157 mV, in raw and pasteurized milk cheese, respectively, in about 6 mo. The cheese in the pH 5.4 to 5.6 range did not allow a reliable initial estimation of Eh, since the dye in some areas of the cheese was completely reduced and in others fully oxidized. Curing up to 10 mo. resulted in the nonreduced areas becoming more reduced.

In the platinum electrodes series, which involved 16 lots of cheese with two to four electrodes imbedded in each unit, the initial pH of the cheese ranged from 5.3 to 5.8. The initial Eh range was +300 to −300 mV. The average Eh of cheese which developed good flavor on curing was −104 mV initially, −90 mV after 1 mo., and −217 mV after from 5 to 6 mo. Cheese in which the Eh differed significantly from these values and trends developed acidy, fermented, rancid, or oxidized flavors.

*Supported by funds from the American Dairy Association and from the Ohio Dairy Products Research Fund.

M30. Crystalline rennin in Cheddar cheese manufacture. P. M. LINKLATER AND C. A. ERNSTROM, University of Wisconsin, Madison.

Unsuccessful attempts to produce good Cheddar cheese with crystalline rennin have led other workers to suggest the cheese-making value of substances in rennet extract other than rennin. This suggestion was investigated by making eight, three-vat lots of Cheddar cheese from pasteurized milk to compare the effects of crystalline rennin, commercial rennet, and the supernatant resulting from the salt precipitation of 85% of the clotting activity from rennet extract. The activity of each rennin preparation was measured on a reconstituted substrate (60 g. nonfat dried milk in 500 ml. 0.01 *M* $CaCl_2$), and standardized so that amounts containing equal clotting activities were added to each vat. The cheese, cured at 50° F., was of good quality, and no significant differences were apparent between treatments when graded organoleptically at 2, 9, and 36 wk. However, small but consistent differences in soluble nitrogen content were noted. Cheese made with crystalline rennin yielded the highest soluble nitrogen values, and that made with the supernatant the lowest.

It was found that good-quality Cheddar cheese could be produced with crystalline rennin and that other substances in rennet extract did not seem important to the cheese-making process.

M31. Coagulation of milk for cheese-making by ester hydrolysis. D. D. DEANE AND E. G. HAMMOND, Iowa State College, Ames.

A number of compounds which slowly hydrolyze to produce acid were tested for their ability to form a milk coagulum suitable for making cheese. Of these (including anhydrides, esters, lactones, and lactides), D-glucono-delta-lactone and racemic lactide were most suitable for making cottage cheese from fresh or reconstituted 9.0% solids-not-fat (S.N.F.) skimmilks. D-glucono-delta-lactone, at 12% of the per cent SNF, produced a pH of 4.6–4.7 after cooking. Coagulation rate depended upon rate of hydrolysis which, in turn, was affected by temperature; coagulation time varied from 15–16 hr. at 20° C. to 3 hr. at 35° C. Setting temperatures above 40° C. caused immediate matting after cutting. A lactide level of 9% of the SNF gave a coagulation time of approximately 2 hr. at 25° C. and 1 hr. at 30° C., and a final pH of 4.5–4.6. The pH at cutting (4.7–5.0) depended upon the compound and setting temperature, with the pH being highest when D-glucono-delta-lactone was added at setting temperatures above 30° C. Rennet and/or $CaCl_2$ improved the process. Normal cooking procedures were used. Yields were normal. The curd was bland and kept well.

M32. Formation of carbonyls during ripening of Limburger cheese. S. L. TUCKEY, JOHN COLMEY, AND YASUO AZUMA, University of Illinois, Urbana.

The purpose of the investigation was to determine changes in the number of carbonyl compounds during the various stages of ripening of Limburger cheese. Answers to the following questions were sought: 1. What car-

bonyls are present in fresh curd? 2. Do carbonyls increase in number and concentration during ripening? 3. Can flavor of the cheese be correlated with the presence or absence of specific carbonyl compounds? The procedure used for analysis and extraction of carbonyls from the cheese at weekly intervals was essentially that described by Harper. The neutral carbonyl compounds and keto-acids were chromatographed at 2,4-dinitrophenylhydrazine derivatives. Identification was made by comparing the R_f values of the derivatives of the unknown compounds with those of known compounds. Solvents used for the acidic and neutral carbonyls were, respectively: butanol—0.5 *N* ammonia-ethanol (70-20-10 V/V); and cyclohexane and 5:1 dimethylformamide and pyridine.

Results of the study demonstrated that: 1. Almost all of the carbonyl compounds identified in one-day cheese are also present in cheese aged 12–16 wk. 2. Carbonyl compounds do not appreciably increase in concentration during aging. 3. Carbonyls tend to be cyclic in their presence; that is, individual compounds may be present one week, but absent the following week. 4. Flavor of the cheese was independent of the carbonyl compounds.

M33. Ultrasonic energy attenuation in Cheddar cheese and the effect of ultrasound on the ripening process. A. Federer and W. C. Winder, University of Wisconsin, Madison.

Small disc and cylindrical barium titanate ceramics were used as ultrasonic probes for a study of ultrasonic energy attenuation in Cheddar cheese. When ultrasound passed through close-textured Cheddar cheese for a distance of approximately 2.5 in., the intensity decreased to 50% of the original value. Sound transmission increased with increasing firmness of the cheese, and decreased because of sound scattering in open-textured cheese. The presence of a standing-wave pattern within cheese was revealed during treatment at 400 Kc. The greatest ultrasonic activity and heat formation were found at the antinodes of the standing-wave pattern.

The most favorable acceleration of ripening was obtained when cheese was treated at 1,200 Kc. between two barium titanate transducer elements which were in direct contact with the cheese. A treatment of 90 sec. at a transducer driving power of 300 w. per pound of cheese was shown to be most favorable for series circuit operation of the transducer elements. For parallel circuit operation, a duration of 180 sec. and a driving power of 250 w. produced the best results. Ultrasonic treatment applied in two installments to the cheese, at one and 20 days of age, resulted in a beneficial cumulative effect on the ripening process.

M34. Correlation between the iodine and thiocyanogen numbers of milk fat. V. N. Krukovsky, R. A. Place, S. R. Toole, and R. W. Watson, Jr., Cornell University, Ithaca, New York.

This study was undertaken to determine ways of producing highly palatable milk products with longer shelf life. It revealed that, irrespective of the roughages fed to the cows, there was a highly significant correlation (+0.73) between the iodine and thiocyanogen values of the milk fat. There was no relationship between the degree of unsaturation of fat and susceptibility of fresh milk to oxidized flavors. The iodine and thiocyanogen values of milk fat produced by Holstein cows fed corn silage and legume-grass hay, in ascending and descending orders, respectively, for seven days first and then hay alone for an additional four days, were 33.77 ± 4.42 and 30.15 ± 3.73. The corresponding values for the milk fat produced by Jersey, Brown Swiss, Holstein, and Ayrshire cows on oats silage + very early permanent pasture ration on one hand, and corn silage + alfalfa hay on the other were as follows: Iodine values: 34.03 ± 2.22 ± 41.63 ± 1.74, 40.56 ± 1.16, 39.31 ± 1.58, and 27.70 ± 3.57, 30.25 ± 2.60, 28.63 ± 2.03, 31.41 ± 7.20; thiocyanogen values: 31.12 ± 2.39, 38.29 ± 1.63, 36.58 ± 1.00, 34.87 ± 1.57 and 25.06 ± 2.85, 27.53 ± 2.75, 26.86 ± 2.38, 29.48 ± 6.98.

M35. The monoglyceride content of butter. R. G. Jensen and A. H. Duthie, Storrs Agricultural Experiment Station, Storrs, Connecticut.

Butterfat was obtained from butter by the silica gel extraction method. Total monoglycerides were estimated in the butterfat by isomerizing the 2-monoglycerides to the 1-isomer with perchloric acid, oxidizing the 1-monoglycerides to formaldehyde with periodic acid, reacting the formaldehyde with chromotropic acid, and measuring colorimetrically the purple color development. Interference due to glycerol and lactose was ont experienced, since these are retained in the silica gel. Also, diacetyl and 2,3-butylene glycol which yield acetaldehyde when oxidized by periodic acid did not interfere. Monoglycerides added at the level of 0.1 m*M* per 100 g. of butter were recovered quantitatively. Average total monoglyceride content in 20 samples of butter was 0.15 m*M* per 100 g. of butter, and ranged from 0.08 to 0.62. Three of these samples with an old cream flavor defect had a monoglyceride content greater than 0.2 m*M* per 100 g. butter. Sixteen other samples contained 0.15 m*M* or less per 100 g. butter. Since monoglycerides are probably produced only by lipolysis in butter, the test may have some value for detecting butter made from lipolyzed cream. Monoglycerides are added to margarine as emulsifiers to prevent leakiness and, perhaps, could have the same effect in butter.

M36. Molecular distillation characteristics of butterfat, other food fats, and mixtures of

butterfat and other food fats. E. H. SANDER AND E. W. BIRD, Iowa State College, Ames.

Preliminary data suggested that the amount of a fat mixture which distilled in a pot still might detect adulteration of milk fat. Distillation characteristics of butterfat (150 to 250° C., 1 to 8 hr., 1 to 2 μ), soybean (I.V. 70 to 133; 1 to 2 μ, 250° C., 1 hr.), hydrogenated cottonseed and coconut oils (1 to 2 μ; 250° C; 1 to 8 hr.) were determined. Percentages of oils which distilled and the refractive indices of the whole fats, distillates, and residues were determined. Comparison of distillate quantities of mixtures (1 to 2 μ, 250° C., 1 hr.) with those of milk fats of all seasons showed that coconut oil could not be detected; 15% of domestic animal or vegetable fats could be detected, in winter- and 5% in summer-butterfats. A distillate, less than 62% of the sample, suggests adulteration with domestic animal and vegetable fats. Refractive indices (60° C.) of distillates of mixtures did not detect domestic animal or vegetable fats, but the data suggest that 5% coconut oil may be detectable in winter- and 20% in summer-butterfats. Refractive indices of distillates below 1.4447 indicate that the sample contained coconut oil.

M37. Colorimetric determination of fat in milk and the saponification number of a fat by the hydroxamic acid reaction. I. KATZ, M. KEENEY, AND RICHARD BASSETTE, University of Maryland, College Park.

Further work has been done on the hydroxamic acid reaction as presented by Bassette and Keeney (*J. Dairy Sci.*, **39**: 910. 1956.) Minor modifications of the original procedeure have been made so as to further increase the precision and accuracy of the test. The Mojonnier method for fat determination, and the A.O.A.C. method for the determination of saponification numbers, were used as standards. Forty-eight samples of raw milk, obtained from individual cows with fat percentages varying from 2.48 to 6.83, and 48 samples of mixed herd homogenized milk, with fat percentages varying from 0.02 to 6.80, prepared by blending skimmilk and 12.0% cream, were used in the fat determination study. Thirty-two samples of fat, with saponification numbers ranging from 192 to 254, prepared by blending coconut fat, butterfat, and commercial hydrogenated vegetable fat, were used in the saponification number study. Statistical analysis of the data revealed the following standard errors of estimate for the hydroxamate tests: (a) raw milk from individual cows, 0.15; (b) homogenized mixed herd milk, 0.06; (c) saponification number, 2.34.

M38. Free butyric, caproic, and caprylic acid production during lipolysis.* W. J. HARPER AND I. A. GOULD, The Ohio State University, Columbus.

Analyses were made for the amount of free butyric, caproic, caprylic, and total free fatty acid in milk by the silica gel extraction and separation procedure during the development of rancidity. In one series, raw milk was combined with pasteurized homogenized milk in a ratio of 9:1. In another series, raw milk was homogenized at 2,000 p.s.i. Organoleptic and fatty acids analyses were made initially and after varying periods of storage at 40° F.

The fresh raw milk had acid degrees of 0.7–1.0, and butyric, caproic, and caprylic acid contents of 0–0.6 μM/100 ml., 10.2–12.4 μM/100 ml., and 28–36 μM/100 ml., respectively. When the rancid flavor was first detected, the raw-homogenized milk mixture possessed an acid degree of 2.0, and butyric, caproic, and caprylic acid contents of 10.6 μM/100 ml., 22.2 μM/100 ml., and 40.2 μM/100 ml., respectively. At the rancid flavor threshold for the raw homogenized milk, the acid degree was 2.3 μM/100 ml., and the butyric, caproic, and caprylic acid values were 13.8 μM/100 ml., 18.4 μM/100 ml., and 37.8 μM/100 ml., respectively.

In the raw homogenized milk mixture, free butyric acid did not increase with an increase in the acid degree over 3.5; whereas, in the raw homogenized milk, the butyric acid increased directly with increasing acid degree.

M39. Isolation and characterization of phospholipids from dried whole milk and dried buttermilk. S. M. HUSAINI, H. W. SPRECHER, AND A. M. SWANSON, University of Wisconsin, Madison.

Since dried whole milk develops an oxidized flavor faster than dried buttermilk, it was assumed that their phospholipids may not be identical. Lipids were extracted from both these products with a slightly modified Mojonnier fat extraction procedure. Phospholipids were separated by precipitation with acetone at 5° F. and fractionated into lecithin, cephalin, and sphingomyelin by adsorption on magnesium oxide. Differences were found in physical and chemical properties of the fractions. The data indicate differences in composition of the phospholipids from the two sources.

	Buttermilk phospholipids	Whole milk phospholipids
Phosphorus	3.86%	2.5%
Nitrogen	1.9%	0.9%
Solubility	Soluble in ethyl ether, petroleum ether, ethanol chloroform, and water.	Soluble in ethyl ether, petroleum ether, and ethanol, but less soluble in water.
Melting point	60° C.	49° C.

* Supported by USDA Hatch Funds through the Ohio Agricultural Experiment Station and by the Ohio Dairy Products Research Fund.

M40. A new and simple method of estimating the phosphatide content of milk lipids. G. R. GREENBANK AND M. J. PALLANSCH, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

A simple method of estimating the phosphatide content of lipids extracted from cream by the Mojonnier method has been developed. It is based on the formation of a brown color in the lipid material when it is heated. A standard curve is prepared by plotting the phosphatide content, as measured by analysis of a large number of samples for total phosphorus against the optical density of the same samples when heated under controlled conditions. The phosphatide content of unknown samples is measured from their optical density by reference to the standard curve. This colorimetric method may be used for qualitative or quantitative purposes. The results are not as exact as the chemical method, but the apparatus required is much simpler and the time is much less, especially if a large number of samples are run.

M41. Migration of the phosphatides in the processing of dairy products. G. R. GREENBANK AND M. J. PALLANSCH, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

The effect of changes in the fat globule membrane on the processing and storage of dairy products is not well understood. This work shows the migration of phosphatides during the pasteurization, homogenization, and concentration of whole milk. Homogenization of whole milk causes a migration of the phosphatides from the fat globule membrane to the skimmilk, with a maximum effect in the range from 1,000 to 3,000 p.s.i. At 8,000 p.s.i. the phosphatide content of the separated cream is equal to that of the cream from untreated whole milk. Homogenization of 50% concentrate at high pressure causes a physical change in the fat to such an extent that centrifugation at ca. 20,000 g. for 1 hr. gives almost no cream layer. The relationship of these data to published observations on the development of flavors in dairy products is discussed.

M42. The lipids of the fat-globule membrane of bovine milk. M. P. THOMPSON, J. H. BRUNNER, AND C. M. STINE, Michigan Agricultural Experiment Station, East Lansing.

The fat-globule membrane is a protein-lipid complex. The purpose of this study was to isolate and classify the lipid materials constituting the complex. The native membrane material was obtained by churning five-times-washed, unpasteurized cream. The free-fat was removed by another wash at 0° C. Lipids were extracted from the complex with 35% EtOH in Et_2O, followed by several extractions with 30° C. Et_2O. The combined extracts were evaporated to dryness in vacuo. Phospholipids were removed by precipitation with acetone. The high-melting triglycerides were crystallized from absolute ethanol at 22° C. The ethanol soluble fraction was resolved chromatographically on a silicic acid column by stepwise elution with Et_2O in petroleum ether. Identification of the lipid fractions was based almost exclusively upon infrared spectophotometric analyses, which indicated that the complexed lipids consisted of 43.5% phospholipids and 44.6% high-melting triglycerides. The ethanol soluble fraction was composed of squalene, cholesteryl esters, and free cholesterol.

M43. An improved centrifugal test for fat stability applied to evaporated milk—estimation of homogenizer performance and fat stability in storage. S. J. PEARCE, Carnation Company, Van Nuys, California.

As the trend in industry toward sterilization processes yielding evaporated milk of lower viscosity continues, the problem of fat separation becomes more serious. In this connection, the reduction of fat globule size is important. Recently, there has been a substantial improvement in homogenizer valve design and an increase in allowable pressures, which aid in reducing globule size. For evaluation of these factors, a centrifugal test has been developed to provide an index which (1) can be used to estimate the fat stability in storage, and (2) can be related to homogenization pressure and valve design. Innovations of this technique concern utilization of gravitational forces equivalent to moderate storage time at 100° F., and efforts to approximate age-thinning before testing. The test has also been applied to unsterilized milk, to demonstrate fat stabilizing effects of sterilization and for the estimation of homogenizer performance. Data will be presented showing application of test to the evaluation of homogenizer performance, and correlation with fat separation in storage. An attempt has been made to relate storage stability of milk fat to visual estimates of globule size and resistance to centrifugal separation. Finally, a comparison will be made between this technique and other methods.

M44. Development of a high-efficiency homogenizer valve. C. C. LOO, Carnation Company, Van Nuys, California.

A homogenizer valve designed on the principle of cavitation (the almost instantaneous generation and collapse of minute vapor cavities) was tried extensively in pilot plant as well as in production scales, with exceptionally good results. Fresh milk was satisfactorily homogenized at from 700 to 1,000 p.s.i. At 2,400 p.s.i. evaporated milk of relatively low viscosity was rendered almost completely free from fat separation after 90 days of storage at 80° F. During the course of its development, difficulties were encountered in the choice of valve material, the suppression of vibration, and the simplicity of design. The problem on material was successfully solved by the use of 75 ST or 61 ST aluminum alloy, which is

strong, easy to fabricate, and can be anodized to create an extremely wear-resistant surface. The vibration problem was solved by building in the valve a simple damper, which also keeps the valve components in alignment. The valve contains replaceable wearing parts, which may be easily reconditioned for further usage.

M45. Relative cleanability of various stainless steel finishes. O. W. KAUFMANN, T. I. HENDRICK, I. J. PFLUG, C. G. PHEIL, AND R. A. KEPPELER. Michigan State University, East Lansing.

The relative cleanability of 18-8 Type 302 stainless steel having a 2B, 3, 4, and 7 finish was determined under carefully controlled conditions of soiling with skimmilk inoculated with *B. globigii* spores. Relative cleanability was determined after rinsing with water (T2), rinsing and washing with detergent (T3), and rinsing, washing, and sanitizing with chlorine solution (T4). Cleanability was measured by the direct surface agar plate (DSAP), standard swab contact, and direct agar contact methods. The first procedure detects the number of organisms actually remaining on the surface of the material being tested, whereas the latter two determine the number removed from the surface.

In reference to any one treatment (T2, T3, or T4) an analysis of variance indicates no significant difference among the finishes with respect to their cleanability, when tested by the DSAP method. Using the Duncan Multiple Range Test to group the data, no difference was noted at the 5% significance level in the cleanability of the finishes regardless of the bacteriological test procedure used or the cleaning treatment employed.

M46. Extent of psychrophilic bacterial growth in manufacturing-grade, bulk-tank milk. W. S. LAGRANGE AND F. E. NELSON, Iowa Agricultural Experiment Station, Ames.

Samples of manufacturing-grade, bulk-tank milk were obtained at various seasons of the year from over 230 producers at four Iowa creameries. Psychrophilic plate counts were made at 5° C. for seven days, while the standard plate counts (SPC) were incubated at 32° C. The resazurin reduction test was also run. The psychrophilic and SPC on the samples ranged from less than 30,000 to 200,000,000. In many instances, the psychrophilic count exceeded or was in the same range as the SPC. Inadequate cleaning and sanitization of the dairy utensils and bulk tanks were the primary cause of both high standard and psychrophilic counts. In many cases where disagreement between the SPC and resazurin test was found, the psychrophilic count was in the same range as the SPC.

M47. Lipolytic yeasts in cream from dairy farms. E. R. GARRISON, Arkansas Agricultural Experiment Station, Fayetteville.

The lipolytic yeasts in cream from dairy farms was investigated over a 2-yr. period, and the cultures isolated were identified. About 70 samples per month were obtained from producers' cream and plated on potato dextrose agar, with incubation at 25° C. for four days. From ten to 25 contiguous yeast colonies were picked from a plate of suitable dilution from each sample and spotted on a Nile blue sulphate medium containing dispersed fat. After four days of incubation at 25° C. the inoculations were examined for lipolysis. One lipolytic culture from each cream sample that showed lipolytic colonies was retained for study.

Fat-splitting yeasts were found in 46% of the 1,680 cream samples examined. Of the 21,309 yeast colonies studied, 13.7% hydrolyzed fat. During the winter, about 20% of the colonies isolated were lipolytic, whereas during the summer 10% were of this type. The incidence of lipolytic yeasts in cream declined as the titratable acidity of the cream increased.

The 400 cultures studied were classified by the Wickerham technique into eight species in the genera *Candida, Cryptococcus,* and *Rhodotorula,* with three cultures unidentified.

M48. A temperature for the pasteurization of milk with no intended holding time. L. D. WITTER AND JOSEPH TOBIAS, University of Illinois, Urbana.

Micrococcus sp. MS 102 was used as the test organism in the determination of the temperature to which milk must be heated with no intended holding time in a DeLaval plate heat exchanger, Type P5, to assure a bacterial destruction comparable to the accepted pasteurization standards. Inoculated milk, sampled just before entering the pasteurizing section, was laboratory-pasteurized at 155° F. for 30 min. to establish the degree of bacterial destruction desired. To provide a safety factor, 155° F. was used instead of 143° F. At the immediate exit of the pasteurizing section, the milk temperature was taken by a thermocouple inserted in the line, and bacteriological samples were obtained by a sampling device which allowed for almost instantaneous cooling of the milk. A final milk temperature of 187° F. with no intended holding time was found to cause a bacterial destruction comparable to that of the laboratory pasteurization. The study is now being repeated, using ice cream mix.

M49. A direct microscopic method for detecting antibiotic activity in milk. B. J. LASKA, Florida Agricultural Experiment Station, Gainesville.

The method is based on the principles that antibiotics inhibit bacterial growth and that some lactic streptococci undergo abnormal cell enlargement when exposed to certain antibiotics. To perform the test, 9.8 ml. of raw milk are pipetted into a sterile, screw-cap test tube

and heated at 80° C. for 3 min. The milk is cooled at 37° C. and 0.2 ml. of a 1–10 dilution of a rapidly growing *Streptococcus thermophilus* culture is added per tube. The tubes are inverted five times to mix the contents and incubated at 37° C. for 60–90 min. A control using antibiotic-free raw milk is used for comparison. Methylene Blue stains are prepared according to the Breed microscopic method. The average clump count of five fields is determined and compared to the average clump count for the control. An average clump count below 50% of that for the control indicates definite inhibition. Abnormal enlargement of cells is another indication of antibiotic activity. Using a 90-min. incubation time, 0.025 units/ml of penicillin, 0.2 μg/ml of aureomycin, and 0.2 μg/ml of terramycin can be detected in heated milk samples. Amount and age of inoculum, and length and temperature of the incubation period, affect the sensitivity of the test.

M50. Interaction among pure strains of lactic streptococci isolated from multiple strain cheese starters. V. W. GREENE, Southwestern Louisiana Institute, Lafayette.

The component organisms of several commercial lactic starters were isolated and differentiated biochemically and morphologically. By propagating representative cultures singly and in combination with each other, marked examples of both stimulation and inhibition could be demonstrated with regard to acid production, speed of dye reduction, and oxygen uptake. Stimulatory or inhibitory response was not a unique function of any specific isolate, but depended on the combination employed, the conditions of the trial, and the metabolic reaction being studied. The propagation history also influenced the subsequent associative action of these organisms, newly isolated cultures being more active than those which had been carried for several transfers as pure cultures in the laboratory.

The use of semipermeable membranes as a means of propagating these organisms in pure culture and still in combination with each other is described and evaluated.

M51. A study of potential causes of culture failures in cheese factories.* W. K. MOSELEY AND R. L. WINSLOW, W. K. Moseley Laboratory, Indianapolis, Indiana.

To investigate the relative importance of bacteriophage, inhibitory substances, and sanitizing agents as causes of cheese culture failures, samples of cheese milk, starter, whey, and air-exposed preheated whey ("air") were analyzed. The samples represent 101 culture failures in 91 cheese factories in 20 states and 23 normal vats of cheese in 20 factories in ten states.

Bacteriophage was found in 93% of the failures and 74% of the nonfailures; inhibitory substances in cheese milk from 27% of the failures and 10% of the nonfailures; substances causing a positive test for available chlorine in cheese milk from 44% of the failures and 10% of the nonfailures; and quaternary ammonium compounds (QAC) were not found in any of the cheese milk samples. Bacteriophage was determined by screening bacterial-free filtrates of the starter, whey, and "air" in litmus milk against 16 different single strains of *Streptococcus lactis* and *Streptococcus cremoris*, followed by plaque plate confirmations. Inhibitory substances in cheese milk were determined by the Neal-Calbert procedure and the Cerny-Morris modification of the test for residual penicillin. Tests for chlorine and QAC in milk are in *Standard Methods for the Examination of Dairy Products.*

M52. A simple method for eliminating and controlling bacteriophage in lactic starters. R. E. HARGROVE, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Studies on effects of various salts on lactic streptococci and their bacteriophages showed that the phages did not proliferate in skimmilk containing 2% sodium prosphate ($NaH_2PO_4 \cdot H_2O$:Na_2HPO_4::3:2; pH 6.5), whereas the bacteria grew normally. Thus, a simple and effective means has been developed for freeing lactic bacteria of phage and for maintaining phage-free starters. Sterile concentrated phosphate solution was added aseptically to sterilized milk for mother starters and to fresh skimmilk for bulk starter, to give a 2% concentration. Then, the milks were steamed for 15 and 30 min., respectively, an essential procedure.

Nine lactic strains having different phage sensitivities grew normally in phosphated milk inoculated with homologous phage to titers of 10^7 to 10^8. The titers (plaque) decreased markedly and the cultures were free of phage after three or four transfers. Activity of 14 lactic strains and four commercial starters was maintained during repeated transfer. Growth of 12 *Leuconostoc* strains was not inhibited. Normal development of acid, texture, and flavor occurred in cheese made experimentally with treated bulk starters initially inoculated with single strains and homologous phage.

M53. Propagation of lactic starter cultures in sterile canned skimmilk. H. K. ZIMMERMAN AND J. B. MANN, Carnation Company, Van Nuys, California.

Skimmilk with added NFDM has been tested for the absence of inhibitory material and sterilized and canned aseptically. The resulting product was marketed in 1958 as a medium for the propagation of lactic starter cultures.

* This work was done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946, and is supervised by the Eastern Utilization Research and Development Division.

Product made in this manner is of extremely good quality insofar as color and flavor are concerned. Reflectance data show less color in the sterile product than in skimmilk heated to 200° F. for 30–60 min. Flavor of the new product is comparable to that of skimmilk prepared in the conventional way for use as mother culture medium. The latter point is important because absence of the typical "sterilized" flavor is essential, in order to evaluate cultures for flavor. Skimmilk sterilized in conventional in-can sterilizers is not acceptable for use as mother culture medium, insofar as flavor is concerned.

Marketing surveys have shown the primary reasons for acceptance of the new product to be the savings in time realized in the preparation of mother cultures and the more uniform and active cultures which result. The new product has been used for manufacturing buttermilk, cottage cheese, Cheddar, and other hard cheeses, cream cheese, sour cream, and margarine.

M54. Further studies of the continuous propagation of lactic cultures. B. J. LISKA, E. L. FOUTS, AND H. H. WILKOWSKE, University of Florida, Gainesville.

Continuous propagation of lactic dairy cultures was studied on a laboratory scale, using a 1,000-ml. stainless steel beaker, with a glass cover, for the fermenter. An agitator and electrodes were mounted through the glass cover. The pH within the fermenter was controlled by regulating the flow of incoming milk. The overflow from the fermenter, at a pH of 5.3, entered a glass holding tube. The level of partially fermented milk in the holding tube was controlled by the use of an electronic level control which operated a peristaltic action pump at the discharge end. The pH and titrable acidity of the fermented product discharged from the holding tube depended on the volume of the holding tube. A volume of 1,500 ml. in the holding tube yielded a fermented product with a pH of approximately 4.6. The through-put rate was affected by temperature, pH in the fermenter, and heat treatment of the milk used. Several trials using 11% reconstituted nonfat dried milks, heated at temperatures from 175–200° F. for 30–60 min.were successful for a 2-wk. period at 70–72° F. Coliform contamination was not a problem in the fermentation. The end-product from the fermentation usually had the aroma and flavor of cultured buttermilk. Several problems were encountered which will be discussed.

M55. A storage study of a lyophilized and a frozen lactic culture. G. H. RICHARDSON AND H. E. CALBERT, University of Wisconsin, Madison.

Interest in the use of frozen cultures has prompted a comparison of their activity with that of lyophilized cultures under similar storage conditions. An active strain of *Streptococcus lactis* was carried in sterile skimmilk, using 1% inocula. The Horrall-Elliker test, with slight modification, was used for determining culture activity. After 12 hr. of incubation at 32° C. a culture activity of 0.44 was found. Then, part of the culture was frozen in a dry-ice-alcohol bath and the remainder was lyophilized. Both were tested for activity and stored at −28° C. Freezing alone did not reduce the activity of the culture, but lyophilization lowered the activity to 0.41. The activity of the lyophilized culture was 0.37 and 0.34 and that of the frozen culture 0.31 and 0.25, at the end of 3 and 6 mo. of storage. However, when samples of the stored cultures were inoculated at 1% into sterile skimmilk and incubated at 32° C. for 16 hr., titratable acidities for the lyophilized and frozen cultures were 0.92 and 0.85% respectively, indicating good acid production for the less active frozen samples after prolonged incubation.

M56. New type pasteurizing, deodorizing, and concentrating equipment for use in cheese manufacture. F. P. HANRAHAN, H. E. WALTER, AND A. M. SADLER, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Research on production of pasteurized, deodorized, and concentrated milk for experimental manufacture of cheese is in progress. Milk with standardized flavor is produced by removing volatile feed and weed flavors.

The flexibility of experimental equipment permits operation over a much wider range of heating and vacuum conditions than is possible with present commercial equipment designed for operation under relatively fixed conditions. The pasteurizing portion can be operated from 161° F. with 15-sec. holding, to 195° F. with no holding. The vacuum cooling or condensing portion can be operated from 90 to 195° F., with a capacity of 5,000 lb/hr. The milk either is pasteurized by steam injection, held 15 sec., and then treated in the vacuum chamber where condensed steam and volatile flavors are removed, or is pasteurized by plate-surface heating to 195° F. and then treated in the vacuum chamber where water and volatile flavors are removed with boiling temperature as low as 90° F. The latter removes approximately 10% of the water, which increases cheese vat capacity and concentration of whey by 10%, a substantial economic gain. Satisfactory Cheddar cheese has been made from milk processed by both methods.

M57. Effect of modification of composition on the firmness of pasteurized process cheese spread. N. F. OLSON AND W. V. PRICE, University of Wisconsin, Madison.

The composition of pasteurized process cheese spread was modified to determine the effects of variation of moisture, fat, and replacement of cheese solids-not-fat with either

lactose or nonfat dried milk (NFDM). Dariworld, a soft ripened cheese, supplied the entire protein portion of the solids-not-fat in the basic formula of the spread. When the same proportion of solids-not-fat to fat was maintained in spreads, variation in the moisture content caused significant changes in spread firmness measured by penetrometer. Variation in the fat content caused no significant changes in spread firmness when the same proportion of moisture to solids-not-fat was maintained. Replacement of cheese-solids-not-fat (cheese-S.N.F.) of spreads with NFDM or lactose decreased spread firmness in direct proportion to the amount of cheese-S.N.F. replaced. A definite relationship was established between moisture/cheese-S.N.F. and penetrometer values of spreads.

M58. The manufacture and ripening of cheese made from reconstituted milk. I. I. PETERS, Texas Agricultural Experiment Station, College Station.

Extra Grade Low Heat nonfat dried milk was reconstituted with tap water (9 + 91), heated to 130° F., and sufficient melted sweet butter was added to give 4% fat in the mixture. The mixture was homogenized at 500 p.s.i., pasteurized at 143° F. for 30 min., rehomogenized at 500 p.s.i., cooled, and made into Cheddar type cheese by a conventional method.

Desirable features observed during manufacture were: no creaming of milk, low fat losses in whey (0.02–0.07%), and little tendency of curd to mat during cooking. Undesirable features consisted in curd lacking elasticity, slow matting of curd, and weak rind in some of the finished cheese.

The ripening of such cheese as observed by flavor and body characteristics after 1, 3, and 6 mo. of ripening at 50° F. was slower in the experimental cheese than in raw or pasteurized homogenized natural milk cheese. The growth rate of lactic bacteria in uninoculated cheese decreased from raw to pasteurized homogenized to reconstituted milk cheese. However, similar lactic counts were obtained with all three types of cheese inoculated with a culture of *Lactobacillus casei*.

M59. Examination of commercial low-heat nonfat milk powder for the manufacture of cottage cheese.* H. E. RANDOLPH AND T. KRISTOFFERSEN, The Ohio State University, Columbus.

Thirty samples of commercial low-heat nonfat dry milk were subjected to bacteriological, chemical, and physical examinations, to determine their characteristics and their suitability for use in cottage cheese manufacturing. The determinations, with their ranges and average values encountered, were: Standard plate count, 15,000 to 75,000/g, av. 39,000/g; direct microscopic count, 28,000,000 to 58,000,000/g, av. 41,000,000/g; psychrophilic count, 1 to 6/ml, av. 1/ml; coliform count, 1 to 2/ml, av. 1/ml; titratable acidity, 0.13 to 0.15%, av. 0.142%; actual lactic acid, 0.019 to 0.098%, av. 0.04%; ash, 7.57 to 8.25%, av. 7.87; calcium, 1.21 to 1.42%, av. 1.30%; nitrogen, 5.18 to 5.82%, av. 5.49%; whey protein nitrogen, 2.52 to 7.05 mg/g, av. 6.11 mg/g; dispersibility (by a centrifugal method), 0.00 to 16.63%, av. 1.78%; clumping, 0.00 to 8.90 g.; av. 173 g.; rennet curd tension, 0 to 84 g., av. 50 g.; and acid-rennet curd tension, 71 to 170 g., av. 135 g.

The majority of the samples contained considerable numbers of "sweet curdling" organisms which were spore-forming rods. All samples supported the growth of lactic cultures adequately.

The characteristics investigated, singly or in combination, did not appear to assess accurately the suitability of the commercial powders for cottage cheese manufacturing.

M60. Application of lyophilization to cheese products: I. Some observations on the characteristics and stability of freeze-dehydrated Cheddar, Brick, Munster, Blue, cream, and cottage cheese. R. I. MEYER AND LOUIS JOKAY, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois.

Efforts to reduce weight and increase shelf life without refrigeration, required for items in military subsistence channels, have renewed interest in the use of lyophilization as a method of dairy products preservations. A number of cheeses, natural and processed, were studied. Cheese was freeze-dehydrated in slices (1 by 4 by ¼ in.) and rods (2 in. long by 7/16 in. diameter). Drying cycles took 10–15 hr. (for rods) and 15–20 hr. (for slices) at a maximum operating temperature of 110° F.

Rehydration to a soft but natural consistency took about 12 hr. for Cheddar, brick, and Munster, about 1 hr. for Blue and cream cheese, and about 5–10 min. for cottage cheese. In general, the flavor of all lyophilized samples was milder than their untreated control. Stability studies have indicated the advantage of inert gas packaging for all products. Where the expected cheese flavor is naturally strong (Blue cheese), or additives (like chives in cream cheese) were used, extension of shelf-life was observed. For special user requirements, freeze dehydration of some cheese products (cream and cottage cheese) holds merit which conventional methods of dehydration have not equalled.

M61. Application of lyophilization to cheese products: II. The development of freeze-dehydrated creamed cottage cheese. LOUIS JOKAY AND R. I. MEYER, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois.

Freeze-dehydrated creamed cottage cheese

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has been studied following the development of a satisfactory processing technique. The product has been produced repeatedly on a laboratory-pilot scale below 2% moisture and reconstitutes to an edible condition in less than 10 min. With firm, large curd to which was applied an 18% fat cultured sour-cream dressing, the product was frozen in a layer of ¾-in. thickness within 1¼ to 2½ hr. Lyophilization was conducted below 1,500 μ pressure, with a maximum plate temperature of 110° F. The drying cycle required from 20 to 24 hr., and the yield was approximately 20%.

Stability data utilizing biochemical and sensory evaluation from taste-acceptance panels, on samples stored at 40, 70, and 100° F. for 1 yr. has revealed sample failures in the following order: 100° F. air-packed (A), 10 wk., nitrogen-packed (N), 14 wk.; 70° F. (A), 30 wk., (N), 48 wk.; 40° F. (A), 48 wk. (N) sample was acceptable after 1 yr.

Data are believed directly applicable to some commercial lyophilization equipment.

M62. Observations on freezing and thawing on the attainment of a satisfactory frozen creamed cottage cheese. A. H. Rishoi, J. A. Riekens, and R. I. Meyer, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois.

Responses to freezing and thawing of uncreamed and creamed commercial cottage cheese in rapidly moving air and in still air, along with variations in storage temperatures and fat content of the cream dressing, were observed. Criteria of evaluation included: (1) proportion of whey drainage after thawing at room temperature, (2) ability to reabsorb whey, (3) general organoleptic characteristics of the thawed products, and (4) effect on curd texture.

When cottage cheese is frozen or thawed in an atmosphere of rapidly moving air, attainment of either condition is much more rapid than in still air; also, the degree of whey drainage is less. Drainage varied from 5 to 10%, by weight, when cream dressings of 32 and 20% fat content, respectively, were employed. Uncreamed cheese drained as much as 20%. Samples with up to 10% drainage reabsorbed whey readily; above this level, reabsorption took place more slowly and less completely.

Products stored at 0 and −10° F. for 9 mo. were slightly less acceptable than fresh creamed cheese. Uncreamed possessed better stability than the creamed product. Although still noticeable, the shattered curd could be held at a minimum when very firm curd was employed.

M63. Lactic acid content of cottage cheese.* W. J. Harper and H. E. Randolph, The Ohio State University, Columbus.

Analyses were made of 75 commercial creamed cottage cheese samples selected over an 8-mo. period, to determine the lactic acid content and its relationship with pH, flavor, and keeping quality. Using an adaptation of the Davidson method, the lactic acid content was found to vary from 0.001 to 0.69%, with an average of 0.19%. No relationship was observed between the lactic acid content and the pH of the cheese, which ranged from pH 4.7 to 5.4. For example, cheeses with a pH of 5.0 were found to vary in lactic acid from 0.01 to 0.47%.

In experimental trials, the lactic acid content of the cheese was found to be dependent upon the initial acidity of the curd, the number of washings, the amount and temperature of the water used in washing the curd, and the length of time the curd remained in the water. Lactic acid levels below 0.1% were associated with flat-flavored cheese and lower keeping quality; whereas, values over 0.3% were associated with "sour" flavored cheese.

M64. A method for improving the flavor of creamed cottage cheese. D. W. Mather and F. J. Babel, Purdue University, Lafayette, Indiana.

Biacetyl contributes considerable flavor to various dairy products, including creamed cottage cheese. However, the biacetyl content of creamed cottage cheese is not well standardized. The following method was developed in an attempt to standardize the flavor. Skimmilk is heated to from 190 to 200° F. and held for 1 hr. It s cooled to 70° F., inoculated with 2% of a pure culture of *Streptococcus citrovorus,* and held at 70° F. for from 18 to 24 hr. Subsequently, it is acidified to pH 4.3 with citric acid and held at 70° F. for from 18 to 24 hr. At this time, the skimmilk contains about 6 p.p.m. biacetyl, but with further incubation it can be made to contain 10 p.p.m. or more. The skimmilk containing biacetyl is used to standardize the fat content of 20% cream to make a 12% creaming mixture. Use of such a creaming mixture results in creamed cottage cheese containing about 2 p.p.m. biacetyl. Creamed cottage cheese with a greater amount of biacetyl can be obtained by using a higher-fat cream and correspondingly more skimmilk in the standardization. Cottage cheese creamed in this manner has a satisfactory flavor immediately after creaming and the biacetyl content increases slightly during storage at 45° F. for 11 days.

M65. Influence of a special creaming mixture on the keeping quality of creamed cottage cheese. F. J. Babel and D. W. Mather, Purdue University, Lafayette, Indiana.

When cottage cheese was creamed with a special creaming mixture containing *Streptococcus citrovorus* and its fermentation products,

* Supported in part by the Hatch Funds through the Ohio Agricultural Experiment Station, Wooster, Ohio.

and also with a regular creaming mixture, it was noted that certain types of spoilage did not occur in the cheese creamed with the special mixture. Experiments were conducted to verify these observations. A culture of *Pseudomonas fragi*, known to produce slime and fruity flavor in cottage cheese, was added to cottage cheese creamed with a regular creaming mixture and the special creaming mixture. The organism was added to both lots in an amount equivalent to 67,000 organisms per gram of cheese. Cheese creamed with the regular creaming mixture developed slime and fruitiness within five days at 45° F.; no defects were noted in the cheese creamed with the special mixture in 12 days. Biacetyl was not detected in the regularly creamed cheese after two days; whereas, in the specially creamed cheese it was slightly higher than its initial value at 12 days. Similar results were obtained when *Pseudomonas putrefaciens* was used as the spoilage organism. The special creaming mixture also had a restraining action on the growth of coliform organisms, but no effect on the growth of *Geotrichum candidum* or *Candida pseudotropicalis*.

M66. Effect of early lactation skimmilk for making cottage cheese and cultures. L. O. Luedecke, L. G. Harmon, and J. R. Brunner, Michigan State University, East Lansing.

Starter cultures were made from blends of skimmilk containing from 0 to 50% colostrum, and cottage cheese was made from blends of skimmilk containing from 0 to 25% colostrum. The term colostrum, as used herein, refers to the milk produced during the first ten days after parturition. The samples propagated as cultures were analyzed for starter activity and curd firmness after starter coagulation. The milks used in making cheese were analyzed for protein content and composition, starter activity, curd firmness at the time of cutting, yield, and characteristics of the finished curd.

Starters prepared from colostrum-skimmilk blends were higher in developed acidity than those prepared from normal skimmilk. Starter activity, in terms of lactic acid production, was essentially normal in skimmilk containing from 3 to 25% colostrum. Lower curd firmness values at the cutting time resulted when early colostrum skimmilk was added to normal skimmilk. No decrease in the yields of curd was observed. The body and texture of the finished curd was normal, but the size and shape were irregular.

M67. A study of the microbial flora of cottage cheese during storage. W. W. Overcast and J. V. Britton, University of Tennessee, Knoxville.

Cottage cheese manufactured under plant conditions was studed to determine possible microbial standards for fresh cheese and cheese stored up to 11 days. Total plate, psychrophilic, coliform, and yeast and mold counts were made on the fresh cheese and again after seven and 11 days. With acidified and chlorinated wash waters, the initial psychrophilic counts have been kept extremely low. However, after 11 days of storage at 40° F. some psychrophilic growth occurred. Coliform counts initially and also after 11 days of storage have averaged less than ten per gram. Considerable variation in initial total counts has occurred and this may be partially influenced by the temperature and time of cooking. Under carefully controlled plant conditions, cottage cheese can be manufactured with a shelf life of at least 15 days, with development of little or no microbial off-flavors or visual defects.

M68. A method to improve the storage life of frozen concentrated milk. D. R. Braatz and W. C. Winder, University of Wisconsin, Madison.

The relatively short storage life of frozen concentrated milk, when held at temperatures above 0° F., has limited commercial production and distribution of this product. Processing studies have resulted in improved keeping quality of 36% total solids concentrate stored at temperatures around 10° F. Prolonged storage life was obtained by forewarming the raw whole milk to a temperature not exceeding 143° F., homogenizing, condensing at a temperature below 140° F., packaging in tinned steel containers, followed by a heat treatment of 155° F. for 25 min., cooling without agitation, and freezing under quiescent conditions. The most important step in this process was the post-condensing heat treatment of the concentrate. However, any agitation after cooling below 100° F., or during the freezing of the concentrate, reduced the beneficial effects produced by the post-condensing heat treatment. It was possible to produce a frozen concentrated milk under these conditions that was acceptable in flavor and body for about 4 mo. of storage at 10° F.

M69. A study of the effect of variations in the initial viscosity of HTST sterilized evaporated milk at the time of aseptic homogenization and subsequent gelling in storage. L. F. Edmondson, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

Whole milks were concentrated to 26 and 32% total solids. Sterilization was accomplished with a small Mallory tubular heat exchanger. Based on pilot sterilization trials, lots of sterilized milks containing selected amounts of $CaCl_2$ and Na_2HPO_4 to vary the initial viscosity and degree of incipient coagulation were homogenized aseptically, canned, and stored at 70 and 100° F. Gelation was followed by viscosity determinations.

The milks containing the most added phosphate, and having the lowest initial viscosity, gelled most rapidly. Control of the initial viscosity within a range approaching incipient coagulation holds promise of obtaining maximum shelf life.

The relationship between homogenization pressure, initial viscosity, protein stability, and homogenization efficiency will be discussed. The use of aseptic homogenization permits the use of higher pressures without troublesome heat instability.

M70. Viscosity studies of concentrated milk, sterilized by the ultra-high-temperature, short-time process. H. K. WILSON AND E. O. HERREID, University of Illinois, Urbana.

The viscosity of concentated milk, sterilized by the ultra-high-temperature, short-time method has been studied in relation to storage at 40, 70, and 100° F. Initial viscosities at 40° F. before sterilization varied from 87 to 137 centipoises, depending on fat content, solids-not-fat, and heat treatment before condensing. Sterilization decreased viscosity. At 40° F. storage the viscosities increased at varying rates, depending on the processing. The rates were greater for the milks that had the more severe heat treatment and had a greater percentage of the serum proteins denatured. Viscosity was least in milk with no preheating, but it developed a strong rancid flavor during condensing. During storage at 70 and 100° F., viscosities increased slowly until first stages of gelation were observed, and then they increased rapidly. In most cases, at 70 and 100° F., the first stages of gelation were noted after from 21 to 35 days. To date, the most satisfactory product has been made by preheating sufficiently to inactivate lipase, followed by concentration and then sterilization with minimum heating and storing at low temperatures but not freezing.

M71. A study of factors related to the storage stability of foam-dried whole milk. ARJEN TAMSMA, M. J. PALLANSCH, AND T. J. MUCHA, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

A study was made of the effect of variations in oxygen level in the package, heat-treatment during processing, antioxidants, recombining of milk, fat level, degree of crystallization of lactose, and percentage of free fat, on the flavor score of foam-dried whole milk during storage.

Low oxygen level (0.1%) and optimum heat treatment of the milk (in excess of the minimum required for pasteurization) markedly improved storage stability. Heat treatment was most effective for storage in the presence of oxygen. A product judged to be satisfactory for beverage use by an expert panel after 6 mo. of storage at 80° F. was obtained by proper control of these two factors. Among the antioxidants tested, lauryl gallate was the most effective.

Products containing fat deteriorated in storage much more rapidly than nonfat products. A dry milk containing 15% fat was more stable than the regular product (26% fat) when stored at low oxygen level.

Results, which agree with those on spray-dried milk from the literature, except the superiority of 0.1% over 1% O_2 for the foam-dried product, indicate lipid oxidation as a primary factor for lowering the score of foam-dried whole milk in storage.

M72. Effects of variations in processing methods on the free-fat and dispersibility of whole milk powder. E. REINEKE AND J. R. BRUNNER, Michigan Agricultural Experiment Station, East Lansing.

Whole milk powders of varying butterfat contents were manufactured in a commercial-sized dryer with selected modifications in the manufacturing procedures. Samples were analyzed for free fat, self-dispersion, and flavor when fresh and again following storage at 33, 72, and 90° F. for periods of 1, 3, 6 mo., and 1 yr. Processing variations included: (a) high and low forewarming temperatures (120 to 300° F.), (b) the use of low fat milk to produce powders containing from 10 to 28% fat, (c) recombining of condensed skim with cream, (d) use of raw and homogenized milk, (e) two-stage homogenization of the condensed milk, and (f) employment of various nozzles and air temperatures.

Free fat varied from 1 to 6% of total fat in the fresh powder; it increased slightly on storage and was lowest in low fat powders. Self-dispersion varied from ½ to 1 g. per 15 g. of fresh powder, but this value decreased with storage.

A typical whole milk powder flavor was noted in all reconstituted samples and this was intensified by storage. Low-fat powders were superior to those of high-fat.

M73. Effect of preheat treatments applied separately to cream and skimmilk on the cooked and oxidized flavors in dry whole milk. C. H. AMUNDSON AND A. M. SWANSON, University of Wisconsin, Madison.

Samples of pooled fresh whole milk were separated at temperatures of 80 and 135° F. Preheat treatments of 145 and 190° F. for 30 min. were applied separately to the cream and skimmilk. The preheated creams and skimmilks were then reblended to give all possible combinations of preheat treatments. Unseparated samples were carried along as controls. The unseparated and reblended samples were then homogenized and concentrated to 40% total solids. The concentrate was dried in a laboratory-type Niro Spray Drier operated with an inlet air temperature of 320-345° F. and an outlet air temperature of 150–160° F. This powder was vacuumized, canned under nitrogen, and samples were reconstituted and presented to a taste panel for evaluation at 4-wk. intervals. The results showed that no particular advantage in antioxidant properties can be expected by the use of separate preheat treatments on the cream and skimmilk. In addition, it was noted that the cooked flavor of the powdered milk never exceeded the cooked flavor of the preheated milk from which it was made.

M74. Some volatile carbonyls from stale dry whole milk. O. W. PARKS, N. P. WONG, AND S. PATTON, The Pennsylvania State University, University Park.

A study of the volatiles obtained on low-temperature vacuum distillation (35–40° C., 15–20 mm.) of reconstituted dry whole milk suggests that carbonyl compounds contribute to the stale flavor of this product. The following volatile carbonyls have been conclusively identified through their 2,4-dinitrophenylhydrazones: formaldehyde, acetaldehyde, acetone, butanone-2, pentanone-2, hexanone-2, heptanone-2, and nonanone-2. Data for identification of various other volatile carbonyls present are being accumulated. Preliminary experiments on raw whole milk have revealed that the majority of carbonyls in dry whole milk occur as a result of heat treatment and storage of this product. In view of the assembled data, the significance of carbonyl compounds in the stale flavor of dry whole milk will be discussed.

M75. A solid in-package oxygen absorbent and its use in dry milk products. W. P. JACKSON AND C. C. LOO, Carnation Company, Van Nuys, California.

Sodium sulfite takes up oxygen to form sodium sulfate when exposed to air and moisture. This reaction can be accelerated by the introduction of cupric ions. The present development makes use of the fact that $CuSo_4 \cdot 5H_2O$ contains two weakly bound water molecules and copper. When anhydrous sodium sulfite and $CuSo_4 \cdot 5H_2O$ are brought into intimate contact, either by grinding or by tableting, a mixture capable of oxygen absorption is obtained. Its rate of reaction varies with the degree of contact. A tablet consisting of 0.5 g. Na_2SO_3 and 0.75 g. of $CuSO_4 \cdot 5H_2O$ in an oxygen atmosphere at 20° C. was observed to react with 44.5 ml. of O_2 in 159 days. Other sulfite compounds, notably those of lithium, potassium, barium, iron, and calcium will react in this mixture. Tablets of this mixture enclosed in porous paper pouches and placed in cans of milk powder will continue to absorb oxygen after the cans have been closed. Tests indicate that whole milk powder packaged with the oxygen-absorbing mixture will retain higher taste acceptance than will powder packaged without it.

M76. Factors affecting the visual method of detecting antibiotics in milk. K. M. SHAHANI, Department of Dairy Husbandry, University of Nebraska, Lincoln.

Previous studies revealed that when a green-turquoise dye was mixed with penicillin preparations and infused intramammarily it appeared in the milk, coloring it green, and thus provided a satisfactory method for the visual detection of the antibiotic in milk. When the dye was infused with terramycin, aureomycin, streptomycin, or polymyxin it was secreted in milk for as long as was terramycin, aureomycin, or polymyxin, but longer than streptomycin. There was a negative relationship between the milk yield of the animal and the number of milkings for which the dye and antibiotics appeared in milk. The age and breed of the animal did not seem to affect the number of milkings for which the dye appeared in milk. During the storage of antibiotic-dye mixtures for several months, the dye exerted no appreciable effect upon the stability of the antibiotics. The dye does not cause any ill-effects upon the milk yield, any irritation to the tissues, or any apparent toxic effects when ingested. When the dye and antibiotic preparation were injected rather than infused, the milk did not possess any green color.

PRODUCTION SECTION

P1. "Galactomannanase" activity of rumen bacteria. P. P. WILLIAMS AND R. N. DOETSCH, University of Maryland, College Park.

A purified galactomannan was prepared from "Jaguar," a commercially produced guar gum. Galactomannan hydrolysis was studied by viscosimetric techniques.

Using conventional techniques of rumen bacteriology, 18 pure cultures of galactomannanase-positive bacteria were isolated and characterized. Predominant among these isolates were obligately anaerobic streptococci and diplococci. Some of the latter were quite small, being approximately 0.4 μ in diameter.

Galactomannanase activity was followed viscosimetrically and by determining carbohydrate utilization. The enzyme was purified from rumen liquor, using precipitation and elution procedures. Temperature, pH, and substrate concentration optima have been determined. Counts on galactomannase-positive bacteria in rumen liquor samples were found to be appoximately 4×10^8/ml.

P2. Factors affecting the composition of rumen gas. R. G. KINGWILL, R. A. OPPERMANN, W. O. NELSON, AND R. E. BROWN, Univesity of Illinois, Urbana.

Aliquots of bovine rumen gas were collected continuously and analyzed at intervals during the digestive cycle. Gas composition varied in a regular manner with the time from feedng. The ratio of CO_2: CH_4 was narrowest after fasting (1:1), and was widest (3:1) within 2 hr. after feeding. Following this peak, the ratio narrowed until 4 hr. after feeding, when it widened again to a secondary peak. Thereafter, the CO_2: CH_4 ratio gradually narrowed to that of

the fasting level. Similar patterns were obtained when the ration was either hay and concentrate or concentrate alone. The widest ratios of CO_2: CH_4 were obtained when the ration included roughage. Although terpenes inhibit in vitro methane formation from acetate, the addition of D-limonene to feed to give estimated concentrations of 9×10^{-4} *M*, 1.8×10^{-3} *M*, or 3.6×10^{-3} *M* in the rumen had no effect on in vivo methanogenesis. Continuous sparging of the rumen ingesta with free H_2, to the extent that the rumen gas contained 22% H_2, had no effect on rumen methanogenesis as measured by the ratios of CO_2 to CH_4 in the rumen gas. These latter data do not support the postulate that H_2 is the limiting factor in rumen methanogenesis.

P3. In vivo rumen methanogenesis from labeled substrates. R. A. OPPERMANN, W. O. NELSON, AND R. E. BROWN, University of Illinois, Urbana.

In vivo rumen methanogenesis was studied by analysis of ingesta and gas samples at intervals after the addition of acetate-1-C^{14}, acetate-2-C^{14}, or formate-C^{14}. CH_4 and CO_2 became labeled soon after the addition of acetate-C^{14}. The labeling in CO_2 was greater than in CH_4, irrespective of the position of the label in the acetate. The butyrate fraction of the volatile acidity later became appreciably labeled. The propionate fraction was only slightly labeled. Labeled acetate was detected in the rumen for periods up to 10 hr. Analysis of the data suggests that from 3 to 5% of rumen CH_4 was derived directly or indirectly from acetate. The addition of formate-C^{14} in 1*M* of cold carrier resulted in the rapid labeling of both CO_2 and CH_4. The label from formate-C^{14} appeared in acetate, propionate, and butyrate. Acetate became labeled before the other acids; however, propionate became labeled to a greater extent than did either acetate or butyrate. Labeled formate and its cold carrier were not detected in the rumen after a period of from 30 to 45 min. Free hydrogen was not found during formate decomposition, and the constant specific activity of the added substrate indicates that no formate was generated in the rumen during this period.

P4. Studies on amylolysis in the bovine rumen. P. J. PROVOST AND R. N. DOETSCH, University of Maryland, College Park.

Comparative bacteriological studies were made on a fistulated cow receiving first a normal, high-roughage alfalfa diet and subsequently a diet of flaked corn and alfalfa pellets.

Total bacterial counts (anaerobic incubation at 39° C.) were made on a medium containing rumen fluid, glucose, cellobiose, and starch. The count on the normal diet averaged 2.3×10^8 col/ml, while on the high starch diet the average was 9.7×10^8 col/ml. Subculture into a starch-containing medium showed that approximately 90% of the isolates from the normal diet and approximately 65% from the starch diet were amylolytic. The numbers of amylolytic organisms were, thus, approximately 2×10^8/ml on the normal diet and approximately 8×10^8/ml on the corn diet.

Differences in culturable types of bacteria from the two diets were noticed. Gram-positive rod types represented the largest single culturable group on the corn diet, whereas they did not on the high-roughage diet.

Assays of cell-free rumen liquor from both diets showed considerably greater amylase activity in the liquor obtained from the starch diet.

Some interesting organisms were isolated from the high starch diet. These included Gram-negative cocci resembling the LC coccus and an unusual amylolytic bacterium resembling *Oscillospira quilliermondii*. These were present in significant numbers (10^8/ml).

P5. Effect of minor changes in the ingredient composition of the ration on rumen microbial activity. D. W. CLAYPOOL AND D. R. JACOBSON, University of Kentucky, Lexington.

This study is the beginning of a long-time study to learn the effect of ration ingredient substitution on animal performance. Two fistulated steers and two dry open cows (controls) were used to determine the effect on rumen microbial activity resulting from a single ingredient substitution of corn for barley, or barley for corn and, successively, added 1% salt, the use of a similar but different batch of hay, and added trace minerals. Microbial activity was determined by analyses of rumen volatile fatty acids and volatile fatty acids produced from the dissimilation of cellobiose using the washed cell suspension technique. The beginning rations consisted of 40% alfalfa hay in the long form, 30% of the same alfalfa in ground form, and 30% of either ground corn or barley. These techniques did not reveal any statistically significant changes in microbial activity following any of the above shifts in ration composition. At the beginning of the experiment, at least 14 days were required for microbial activity to become constant. Thereafter, the average concentration and the standard deviation of the mean of short-chain fatty acids in fresh rumen liquor was 84.0 ($\pm$8.4 μM/ml. The fatty acid fraction was composed of 67.0 ($\pm$2.4)% acetic acid, 19.0($\pm$1.5)% propionic acid, 10.5($\pm$2.3)% butyric acid, 2.4($\pm$1.1)% valeric acid, and 0.8($\pm$.7)% higher acids.

P6. Continuous culture for studying mixed rumen microorganisms. DARYL G. STEWART AND R. G. WARNER, Department of Animal Husbandry, Cornell University, Ithaca, New York.

To a 5.5-liter culture, substrate was added at the rate of approximately 450 ml. per hour. The volume was held constant by a float controlling outflow. The culture was stirred slowly and kept at 39° C. Air was excluded by flush-

ing the airtight vessel at the beginning of the experiment. A 2 × 2 factorial arrangement of treatments, with substrates of hay or hay plus grain, suspended in McDougall's salt solution and inocula from two fistulated steers, each fed one of the above substrates, was used and replicated twice. Experiments lasted 24 hr. Samples were taken at 8-hr. intervals until the pH of the culture plateaued. The 16- and 24-hr. samples were averaged to give one value for each experiment. The average content of volatile fatty acids was 15 meq/100 ml., 63% acetic, 21% propionic, 13% butyric, and 3% higher acids for the hay substrate and 15 meq/100 ml., 59% acetic, 24% propionic, 15% butyric, and 3% higher acids for the hay plus grain substrate. The pH values were 6.2 and 5.9, respectively. Counts of *Escherichia coli* were 10^4 per milliliter for both substrates, and counts of amylolytic cocci were 10^6 per milliliter, these counts being in the normal range. The protozoa were highly motile and grew actively.

P7. Effect of concentration on the absorption of volatile fatty acids from the perfused rumen. J. R. STAUBUS, R. E. BROWN, C. L. DAVIS, AND W. O. NELSON, University of Illinois, Urbana.

Three goats were starved 18 hr. and offered 2 lb. of hay and 1 lb. of grain 1 hr. prior to being anesthetized and bled out via a carotid cannula. Each rumen was perfused with homologous blood for from 30 to 60 min. to establish a normal rate of absorption, before the volatile acidity of the rumen was altered by the addition of either butyric or propionic acid. The changes in rumen and blood volatile acidity, glucose and ketone bodies were then measured over an additional 45- to 60-min. perfusion period. The added acid did not cause the rumen pH to fall below physiological limits. Homogeneity of rumen contents was not established immediately on addition of the acids, due to the fragility of the cannulations. The rumen tissue continued to utilize blood glucose throughout the perfusion. An apparent interconversion among acetic, butyric, and propionic acids was observed. An increase in VFA absorption followed an increase in rumen concentration, but the response was not linear. No increase in blood ketones was observed following the addition of propionate, but a significant increase followed the addition of butyrate.

P8. Rumen metabolism of I^{131}-labeled soybean oil. R. S. ALLEN, E. G. HAMMOND, N. L. JACOBSON, AND F. D. HILL, Iowa State College, Ames.

I^{131}-tagged soybean oil (about 1 mc/trial) was poured into the rumen of a steer via a fistula (Trials 1 and 2) or fed with concentrates (Trials 3, 4, and 5). Samples of rumen ingesta were taken at intervals during the first 24 hr.; samples of venous blood and of freshly voided feces were obtained at various times for seven days. Rumen ingesta samples and selected blood and fecal samples were fractionated to permit measurement of I^{131} activity in neutral fats, fatty acids, and iodides. Total I^{131} activity was determined in all blood and fecal samples. Poor distribution of radioactivity in rumen ingesta resulted when labeled oil was administered via fistula, but oral ingestion gave good distribution. I^{131} activity in rumen neutral fats declined sharply; less than half the original activity remained after 4 hr. in most trials. Deiodination of the tagged oil was significant. Very little I^{131} was found in the fatty acids fraction. In Trials 4 and 5, where alfalfa soilage was the major feed, maximum I^{131} activity occurred at 18–24 hr. in the blood and at 32 hr. in the feces. Virtually no lipid I^{131} appeared in the blood, and only traces were found in the feces. In vitro experiments confirmed the in vivo observations.

P9. Investigation of the oxidation-reduction potential of rumen contents. R. L. BALDWIN AND R. S. EMERY, Michigan State University, East Lansing.

The oxidation-reduction potential of rumen contents, removed from several cows at three times during the day, indicates that a close relationship exists between E_h and the metabolic rate. Titration of samples, from which the large feed particles had been removed by centrifugation, with acid and a strong oxidizing agent, respectively, indicated that a linear relationship exists between E_h and pH, and that the metabolic rate of the microflora is reflected by the amounts of reducing substances found. Very little of the reducing capacity of acidified rumen contents was found to be associated with the bacterial cells. The titration curve indicates that the reduction capacity of rumen contents is not due to any one reductive substance. Gas production was the only rumen function that seemed to be affected when the reductive characteristics of rumen fluid were altered by the addition of DPPD, methylene blue, ascorbic, thioglycollate, and cysteine. Addition of oxygen in controlled amounts affected neither the redox potential nor the gas, volatile fatty acid, or ammonia production by rumen contents incubated in vitro. Methylene blue did not affect gas production in formate enrichment cultures of rumen flora.

P10. Methemoglobin and total hemoglobin values in bloated cattle. C. L. MOORE AND A. E. DRACY, South Dakota State College, Brookings.

Fifty-three cases of bloat were produced in 18 cows during the summer of 1958 on irrigated alfalfa pastures. Twenty-six of these cases were slight bloat, 19 moderate bloat, and eight severe bloat. Four of the severely bloated animals died and the other four had to be treated by trocar-cannula or rumenotomy.

Average methemoglobin values in grams per 100 ml. of blood: (normal animals) .539; (slight bloat) .626; (moderate bloat) .457;

(severe bloat) .425. There is no significant difference between normal and bloated animals.

Average total hemoglobin values in grams per 100 ml. of blood are as follows: (normal) 10.68; (slight bloat) 10.20; (moderate bloat) 10.51; (severe bloat) 10.67. Again, there is no significant difference between normal and bloated animals. Consequently, from these data, there is little evidence that the bloat syndrome has any effect upon the formation of methemoglobin.

P11. Carotid arterial blood pressure changes in bloated cattle. C. L. Moore, South Dakota State College, Brookings.

A surgical method for measuring blood pressure has been developed whereby the carotid artery is exteriorized and sutured into a flap of skin. Pressure is then recorded by a sphygmomanometer.

Systolic pressure, heart rate, and respiration rate were obtained from three calves. Diastolic pressure could not be determined whenever the animal had labored breathing. Among 25 cases of bloat, 14 were slight, eight moderate, and three severe. Two severely bloated animals died, while a third was saved by rumenotomy. Systolic pressure increased markedly when the animal was severely bloated.

The average blood pressure, before pasturing, was 175 mm. Hg.; after pasturing: (no bloat) 179 mm. Hg.; (slight bloat) 185 mm. Hg.; (moderate bloat) 196 mm. Hg.; (severe bloat) 242 mm. Hg. The heart rate also had its greatest increase when the animal was severely bloated. The average heart rate per minute, before pasturing, was 90; after pasturing: (no bloat) 110; (slight bloat) 105; (moderate bloat) 101; (severe bloat) 142. There was little change in respiration rate, except that during severe bloat it was extremely labored. The average respiration rate was 38 per minute before pasturing; after pasturing: (no bloat) 72; (slight bloat) 70; (moderate bloat) 69; and (severe bloat) 67.

P12. Effect of bovine saliva and plant mucin on frothing rumen contents in alfalfa bloat. H. H. Van Horn, Jr., and E. E. Bartely, Kansas State College, Manhattan.

The effect of bovine saliva and linseed meal mucin on gas escape from frothing rumen contents obtained from fistulated animals on alfalfa pasture was tested in vitro. In 12 trials, it was found without exception that more gas escaped from the frothing rumen samples to which mucin (0.017 g. solids/ml) or saliva (0.011 g. solids/ml) was added than from the controls. Results for respective treatments in milliliters of gas released were: control, 150; 20 ml. saliva, 170; 20 ml. mucin solution, 176; 80 ml. saliva, 203; 80 ml. mucin solution, 197. Other trials indicated that 160 ml. of mucin or saliva did not release as much gas as the 80-ml. level but more than the 20-ml. level. When nonfrothing rumen contents were used instead of frothing contents, the addition of mucin or saliva did not increase gas escape. When 1 g. of alfalfa saponin was added to 150 ml. water, and air was bubbled through the solution, a stable froth formed. The addition of 50 ml. saliva or mucin solution prevented the formation of a stable froth. These studies support the theory that saliva (mucin) prevents bloating by preventing the formation of a stable froth and by permitting gas to escape from frothing rumen contents.

P13. Roughage capacity and efficiency of feed utilization in dairy cattle. J. B. Stone, G. W. Trimberger, C. R. Henderson, and K. L. Turk, Cornell University, Ithaca, New York.

As a factor in economical milk production, roughage capacity has been studied in 12 experiments at Cornell since 1950. A total of 175 Holstein cows were fed various roughages ad lib. for 20 wk., starting at an average of 60 days after calving. Grain feeding was moderate (averaging 1 lb. per 4.1 lb. FCM).

The average daily dry matter intake from roughage was 26.7 lb., ranging from 12.9 to 35.8 lb. When adjusted for differences in size ($W^{.75}$), the range was from 17.6 to 34.8 lb. The average daily production of FCM was 39.6 lb.; average beginning weight, 1,333 lb.; and average daily change in weight, +0.24 lb.

The 20 experimental weeks were divided into four periods. The average daily intake of dry matter for each period was 25.7, 27.2, 27.3, and 26.7 lb. The daily production of FCM for the same periods was 48.1, 42.0, 36.5, and 31.9 lb. As lactation advanced, roughage consumption did not change appreciably. Analysis of the efficiency of feed utilization showed that, on the average, 100 lb. of FCM required 56.7 lb. of TDN for maintenance and milk; the range for individual cows was from 42.0 to 82.7 lb.

P14. Use of twin efficiency values to measure differences in lactating twins. A. C. Linnerud, J. B. Williams, and J. D. Donker, University of Minnesota, St. Paul.

To measure twin efficiency values, a survey was made of all lactations of twins at the Minnesota station. Records were used only if a comparable record was produced simultaneously by the twin mate. Data were discarded if the total lactational butterfat production was below 100 lb. Records were standardized to 305 days in length, except where cows completed lactations earlier. Data were used from 47 sets of twins and two sets of triplets which completed 156 lactations. Twin efficiency is defined as the number of unrelated animals that one set of twins will replace without loss of statistical precision. Records were divided into categories, which included first-lactation uniform identical twins, uniform identical twins two or more lactations, first-lactation fraternal twins, identical twins on 10–14 hr. milking interval, and identical twins on 8–16 hr. milking

interval. Twin efficiencies were computed for each category on milk production, butterfat production, and butterfat test. Twin efficiencies for first-lactation, uniformly treated identical twins varied from 27 to 32, and 5 to 10 for second or later lactations. Twins on 10–14 hr. milking intervals had efficiency values that varied from 14 to 22, while twins on 8–16 hr. intervals were about half as large. Fraternal twins were about one-fourth the value of similarly treated identical twins.

P15. Solids-not-fat content of the milk of Holstein, Jersey, and Sindhi-Jersey cows in a Louisiana herd. J. L. FLETCHER, USDA, Iberia Livestock Experiment Station, Jeanerette, Louisiana.

Solids-not-fat (SNF) determinations at monthly intervals were carried out on individual milking samples from Holstein, Jersey, and Sindhi-Jersey crossbred cows in the Iberia Station Herd. Use of separate milking samples was prompted by the use of milking intervals of 10 and 14 hr. Determinations were made with the small Watson Pattern lactometer and a portable bath designed for field use. The study extended over 18 mo. and involved more than 1,500 determinations on samples from 75 cows. The data collected appeared worthy of note because the mean SNF content for the Jersey and Holstein cows was much below that usually reported. The unweighted mean of the determinations was 8.80% for Jerseys, 8.37% for Sindhi-Jerseys, and 8.15% for Holsteins. It was found that slightly more than 50% of the samples were below 8.50% SNF. The per cent below 8.50% SNF for Jersey, Sindhi-Jersey, and Holstein cows was 18, 57, and 84, respectively. Other analyses of the data revealed a significantly higher SNF content for samples following the shorter milking interval. Although there appeared to be considerable unexplained variation in SNF content, season and stage-of-lactation effects were apparent. For this herd, the two major sources of variation in SNF were breeds and cows within breeds.

P16. Effect of state of lactation on antibody production to Brucella antigen. H. STRUSS, W. E. PETERSEN, AND B. CAMPBELL, University of Minnesota, St. Paul.

It is known that the udder of the lactating cow will produce antibodies at high levels to infused antigens. However, it is not known if the stage of lactation of the cow at time of intramammary infusion would effect maximum agglutination titer or the time lag required to obtain maximum titer. Six lactating cows that had been milking for eight, nine, 51, 101, 102, and 175 days, respectively, were intramammarily infused with 240 billion cells of a phenol-killed *Brucella abortus* antigen. The infusions were made into one-half of the udder. Milk production was moderately decreased, but returned to near previous production levels. The antibodies in the blood were determined by use of the plate agglutination test. Maximum blood titer was attained by five cows on the tenth day; only one cow's titer was at maximum level on the seventh day. The maximum blood titers ranged from 1–400 to 1–3,200. The milk titer from both the infused and noninfused udder halves was at maximum level, as determined by ring test value of +4, on the seventh day. Individual cow variations were most important. There was no relationship between agglutination titers in the blood or milk to stage of lactation.

P17. Relationship between antibody production in the lactating cow and the time interval between subsequent intramammary infusions. H. STRAUSS, W. E. PETERSEN, AND B. CAMPBELL, University of Minnesota, St. Paul.

It is known that time interval between injections is very critical for antibody production in laboratory animals, but it is not known if time interval is critical in the lactating dairy cow. Six lactating cows were divided into three groups and intramammarily infused at five-, 10-, and 15-day intervals. The antigens consisted of 100 to 200 billion cells of *Salmonella pullorum* that had been twice washed, and 5 ml. of fresh egg white. Each cow was infused in the left front quarter. Antibody response was determined by the plate agglutination test. The maximum titers were in the milk from the infused quarter and the blood serum. This level was 1–10,000. The titers in the untreated quarter were 1–1,000. The time lapse from first to last infusion was 30 days. Five of the six cows attained maximum titer on the 24th day following intramammary infusion, indicating that subsequent infusions do not necessarily cause an increase in agglutination titer. Body temperatures were taken rectally, and indicated a positive relationship between high temperatures and subsequent high agglutination titer. There was no difference in the maximum antibody level that could be attributed to the varied time intervals between subsequent infusions.

P18. Maintenance, proliferation, and biosynthesis of milk constituents by mammary cells in tissue culture. K. E. EBNER AND B. L. LARSON, University of Illinois, Urbana.

Studies on the successful cultivation of bovine mammary gland cells in tissue culture reported last year (*J. Dairy Sci.*, **41**: 727. 1958) have been continued. At present, three strains designated as ILLINI 1581, 1582, and 1583, which were derived from the gland of a prepartum cow, have been in continuous culture for over a year, and cultures from the mammary glands of four additional pre- and postpartum cows have now been proliferating for periods approaching a year. Although the conditions necessary to initiate synthesis of milk constituents have been quite unpredictable, and most attempts have been unsuccessful, data obtained from several short-term cultures (16–18 hr.), derived from the glands of a prepartum and postpartum cow, have indicated synthesis of

lactose and β-lactoglobulin. Human cord serum and an increased oxygen tension appeared to stimulate β-lactoglobulin synthesis. Synthesis of lactose and β-lactoglobulin was also found in several long-term cultures (1½ mo.) derived from the gland of a prepartum cow after treatment with hormones and postpartum bovine serum. Studies are now in progress to define the conditions necessary for the control of growth and synthesis of milk constituents in mammary gland tissue cultures.

P19. Biochemical characterization of bovine mammary cells grown in tissue culture. C. R. HOOVER, E. C. HAGEMAN, K. E. EBNER, AND B. L. LARSON, University of Illinois, Urbana.

Since long- and short-term cultures of bovine mammary tissue have been established in this laboratory, they have been considered to serve as model systems for studying mammary gland functions. To evaluate these systems, it has been necessary to define their characteristic growth and biochemical properties. The cultures of bovine mammary cells that have been maintained possess different metabolic characteristics and physical appearances. Some cultures grow rapidly, whereas others are more deliberate in their mitotic rate. Cortisol in conjunction with some pituitary hormones appears to have an influence on the initial organization of the cells. Generally, the poorer-looking cultures have given the indication of synthesis of milk constituents. Studies suggest that glucose utilization and lactate production are variable and are influenced by many factors including age, cell number, appearance, and frequency of media changes. In contrast to many cells isolated from other sources, there has been no indication that the arginine requirements of the cells are in any way limiting. These and other biochemical studies in progress will be reported.

P20. Anterior pituitary tissue as a stimulator of mammary duct growth. R. P. REECE, New Jersey Agricultural Experiment Station, New Brunswick.

An attempt has been made to determine whether or not anterior pituitary tissue of animals in various physiological states varies in its ability to stimulate mammary duct growth. Three hundred and ninety-four Hooded Norway suckling rats were used in testing anterior pituitary tissue for mammary growth-stimulating properties. Sex of the immature donor rat had little influence on pituitary potency, but pituitaries of mature animals were more potent than those of immature ones. Neither ovariectomy nor the suckling stimulus in the lactating rat influenced pituitary potency. Seven of eight assays on pituitaries from normal rats and rats injected with estrogen showed the latter to be more potent, although the differences were not great. On the whole, anterior pituitaries of animals in various physiological states did not differ markedly in their ability to stimulate mammary duct growth.

P21. Experimental mammary gland growth.* R. C. MOON,** D. R. GRIFFITH, AND C. W. TURNER, University of Missouri, Columbia.

Groups of pregnant and ovariectomized rats of the Sprague Dawley-Rolfsmeyer strain, with an initial weight of 210-140 g., were used. After a 14-day recovery period, the castrated rats received daily subcutaneous injections of estradiol-benzoate (E-B) and progesterone (P) for 19 days, in ratios of 1 μg. E-B to 0-10 mg. P. Each rat was killed on Day 20 after initiation of treatment and the abdominal-inguinal glands removed for determination of deoxyribonucleic acid (DNA). Animals killed on Days 18–20 to pregnancy served as controls. Mean total DNA/6 posterior glands was used as an index of mammary gland growth. Mean total DNA of glands of rats treated with 1 μg. E-B and from 2–10 mg. P did not differ significantly from DNA values obtained with pregnant animals. DNA values for rats treated with 2–10 mg. P were not significantly different when compared with each other. These data indicate that maximum growth of the mammary glands (mean total DNA) of rats is attained with 1 μg. E-B and 2 mg. P, and that increasing the progesterone level to 10 mg. has little, if any, beneficial effect upon growth. Mammary development, under these conditions, is equivalent to that which occurs during pregnancy. Analysis of DNA values suggests that total DNA is a more reliable index of mammary gland growth than DNA/mg.

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** Postdoctoral Fellow of National Cancer Institute.

P22. Effect of recurring pregnancy on mammary gland growth in mice.* HIROSHI WADA** AND C. W. TURNER, University of Missouri, Columbia.

On the base of deoxyribonucleic acid (DNA), mammary gland growth during pregnancy and the effect of recurring pregnancy on mammary growth was studied, employing albino mice bred successively without nursing period. In primipara, total DNA was 2,853 μg. at six days of pregnancy, 4,118 μg. at 12 days, and 5,965 μg. at 18 days. This increase of total DNA shows that mammary gland growth continues during the second half of pregnancy. This observation was rather unexpected, since morphological study of lobule-alveolar growth can not distinguish between additional cell growth and initiation of milk secretion. These data indicate for the first time that cell multiplication

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continues throughout pregnancy. Total DNA was 7,677 μg. in bipara, 7,730 μg. in tripara, and 6,982 μg. in quadripara. Maximum growth of mammary glands was attained in triparous animals. A slight decline in total growth was observed in quadriparous animals. Their age at this time was estimated to be equivalent to the age of cows which begin to decline in yearly milk and fat yields. It was also confirmed that increased mammary gland growth in multiparous animals was more closely related to hormonal stimuli associated with recurring pregnancy and lactation than to increasing body weight.

P23. Effect of oxytocin injections upon the rate of milk flow in dairy cows. E. W. HUPP,* W. W. SNYDER, AND R. C. LEWIS, Michigan State University, East Lansing.

The objective of this experiment was to determine if the rate of milk flow could be changed by the administration of oxytocin. Two trials were conducted, in which 20 I.U. of oxytocin were injected intra-jugularly immediately before milking. In the first trial, seven slow-milking cows were used. There were no statistically significant differences between treatment and control milkings for average or maximum rate of milk flow or for any of the components of the milk-flow curve. In the second trial, seven average- to fast-milking cows were used. In some of the treatment milkings, the average and maximum rates of milk flow were significantly greater than in the control milkings. In some cases, the time to reach a high rate of flow was decreased in the treatment milkings. However, these changes in rate of flow were small compared to differences in rate of flow between cows. These results indicate that in slow-milking cows the rate of milk flow can not be changed by oxytocin injections; whereas, in average- to fast-milking cows there may be a slight increase in the rate of flow.

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P24. Comparison of the intravenous and subcutaneous injections of oxytocin in the lactating cow.* B. N. PREMACHANDRA, G. W. PIPES, AND R. VON BERSVORDT-WALLRABE,** University of Missouri, Columbia.

No information is available so far on the comparative effectiveness of subcutaneous and intravenously administered oxytocin in removing the residual milk after normal milking in the cow. Maximum yields of residual milk have generally been obtained when 10 I.U. of oxytocin has been administered intravenously, and this was confirmed in our investigations. However, in most cases comparable yields of residual milk could be obtained also by injecting 20 I.U. of oxytocin subcutaneously. While 20 I.U. of oxytocin injected subcutaneously caused the second let-down of milk (i.e., after the end of normal milking) in 1½–2 min., 10 I.U. required between 5–6 min., as contrasted to 15–25 sec. when 10 I.U. was injected intrajugularly. Subcutaneous oxytocin injections prevented the decline in milk yield for a considerable period and there was also an increase in the total yield. The higher plane of milk yield was maintained for about four days, even after cessation of treatment. The exact requirements of oxytocin for high producers are not known at present, since the animals available for the investigation were low-producers. Nevertheless, our results indicate that subcutaneous therapy could conveniently replace more cumbersome intravenous injections in removing residual milk.

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P25. Lactogenic hormone requirement for milk secretion.* CLARK E. GROSVENOR,** University of Missouri, Columbia.

Experiments were designed to determine the amount of lactogenic hormone required for the secretion of milk in lactating rats whose endogenous secretion of the hormone had been prevented by Nembutal. Fourteen-day postpartum lactating rats were isolated from their young for 11 hr. Each mother was then anaesthetized and her young replaced and allowed to nurse with the aid of oxytocin. With endogenous lactogen secretion blocked, the amount of milk obtained subsequently with oxytocin after a second 11-hr. isolation period was only 36% of that normally obtained without anesthesia. A single injection of 0.5, 1.0, or 2.0 mg. lactogen, given immediately after the first nursing, restored milk yields to 67, 75, or 75% of normal. However, much individual variation in milk yield resulted with any one level of lactogen. These data suggest: 1) Lactogenic hormone is necessary for the maintenance of milk secretion in the lactating rat; 2) variation in results may indicate that amount of lactogen reflexly released may limit amount of milk secreted; 3) the inability of lactogen alone to restore full milk secretion may indicate that other pituitary hormones necessary for milk secretion are reflexly discharged by the stimulus of nursing or milking.

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** Postdoctorate Fellow of N.I.H.

P26. Effect of five levels of animal fat in calf milk replacers. W. A. OLSON AND J. B. WILLIAMS, University of Minnesota, St. Paul.

Thirty-three, four-day-old dairy calves were used to determine the effects of 0, 5, 10, 20, and 30% stabilized lard in milk replacer diets. Fortified, low heat, dry skimmilk solids made up the remainder of the milk replacer. Each

replacer was reconstituted 1:10 with warm water and fed to all calves at the same rate, based upon 10% of average body weights. The liquid ration was halved at 36 days, and the calves weaned at 42 days.

Average daily weight gains were 0.76, 0.79, 0.85, 0.86, and 0.93 lb. for the 0, 5, 10, 20, and 30% fat diets, respectively. At 28 days, the average daily gains were 0.32, 0.39, 0.52, 0.49, and 0.65 lb., respectively. Dry feed consumption decreased with increasing per cent fat in the liquid diet. Scouring rate was higher with the calves on the fat-free diet, but no significant differences occurred among other treatment groups. The incidence of abnormally high temperatures ($>103.5°$) and average body temperature for the first 2 wk. of the experiment were not significantly different between treatments.

It appears that there is value in the feeding of a 3% animal fat reconstituted milk replacer to calves under 4 wk. of age.

P27. Digestibility of milk fat by the young dairy calf. C. W. GRIMES AND K. E. GARDNER, University of Illinois, Urbana.

Frequent assertions that the calf has limited ability to digest milk fat, plus the common recommendation that milk of high fat content should be avoided, call for more evidence than has heretofore been available. Thirty-two bull calves of different breeds, aged five to 15 days, were placed on six- or seven-day digestion trials in the following groups: I—3% fat milk at 8% of body weight; II—6% fat milk at 8% rate; III—6% fat milk at 5.5% rate; IV—9% fat milk at 8% rate. Aureomycin or terramycin was fed. The fat digestibilities were: Group I—(6 calves) 96.2%; II—(6) 97.4%; III—(4) 95.9% IV—(4) 94.2%. Twelve calves were removed due to scouring during either the preliminary period or the trial. These were by Groups I, II, III, and IV, 1, 2, 2, and 7 calves, respectively. Daily fat intakes averaged 95, 196, 128, and 268 g. for the groups as listed. Protein and dry matter digestibilities were also determined. As determined in this experiment, the digestibility of milk fat by the young calf was very high in milk varying from 3 to 9% fat. The incidence of scouring was highest for the 9% fat milk.

P28. Results of studies concerned with determining the minimum protein requirement of the very young calf. W. A. HARDISON, C. G. NOBLITT, R. W. ENGEL, AND G. C. GRAF, Virginia Agricultural Experiment Station, Blacksburg.

Calves were raised from birth to 8 or 12 wk. of age on liquid diets varying in protein content from approximately 15.5 to 26% protein (DM basis). The diets used were whole milk (control) and whole milk diluted with a 17% sugar solution (12% sucrose and 5% lactose). All of the diets were supplemented with trace minerals, along with vitamins A and D, and were given in two feedings daily at the rate of 10% of body weight. In Trial 1, calves (six per treatment) were fed diets containing 15.5, 17.1, and 25.7% protein. Average daily gains to 12 wk. of age were 0.36, 0.47, and 1.06 lb., respectively. In a second trial, the following diets were compared: whole milk (25.0% protein), diluted whole milk (20.4% protein), and diluted whole milk plus enough sodium caseinate to make it equivalent in protein content to whole milk (26.4% protein). There were four calves per treatment, and over a period of 8 wk. daily gains averaged 1.05 (0.89-1.35), 0.88 (0.78-1.09), and 0.97 lb. (0.71-1.15), respectively. The gains were not significantly different. Additional work is in progress.

P29. Preliminary studies regarding the optimum protein level of milk replacers. C. A. LASSITER, R. M. GRIMES, C. W. DUNCAN, AND L. D. BROWN, Michigan State University, East Lansing.

Forty-eight, two-day-old male calves were used in two trials to determine the minimum amount of protein required in a milk replacer. Replacers were fed which contained 30.9, 24.1, and 15.2% crude protein, along with a 16.5% protein calf starter and alfalfa hay. Body weight gains to 72 days of age indicated very little difference in the gains of calves fed the 30.9, 24.1, 18.7% protein groups, averaging 1.00, 1.05, and 0.99 lb. per day. Growth of calves fed the 15.2% replacer was only 0.90 lb. per day. Weight gains to 49 days of age averaged 0.81, 0.86, 0.77, and 0.73 lb. per day for the four groups in the same order. None of these differences among groups were statistically significant. Crude protein digestibility, nitrogen retention, and per cent nitrogen retained of intake decreased significantly as protein content of the replacers decreased. Blood urea nitrogen and serum nonprotein nitrogen levels decreased significantly as protein content of the replacers decreased, but replacer protein content had very little effect on total serum proteins. Likewise, replaced protein level had very little effect on relative concentrations of serum β- and α-globulins or albumins, but serum γ-globulins tended to increase with decreasing protein level. Results of these studies show that milk replacers should contain more than 15.2% crude protein for optimum growth and health.

P30. Effects of age and diet on the secretion of pregastric esterase in the calf.* J. W. YOUNG, H. A. RAMSEY, AND G. H. WISE, North Carolina State College, Raleigh.

The secretion of pregastric esterase (PGE) was studied in two groups of three calves from 16 through 180 days of age. Group I was fed

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only whole milk, whereas Group II received whole milk initially and was changed by 8 wk. of age to grain and hay. The rate of PGE secretion was determined at frequent intervals by nipple-feeding 5 lb. of nonfat milk, collecting the milk-saliva mixture from the cardial end of the esophagus via a rumen fistula, and measuring the lipolytic activity thereof. Milk fat was used as the substrate. PGE activity (μM of fatty acid/0.2 g substrate) for successive 15-day periods was as follows for Groups I and II, respectively: 113, 114, 113, 112, 108, 101, 104, 101, 110, 98, and 113; 106, 104, 105, 105, 98, 101, 101, 102, 107, 105, and 106. Thus, PGE secretion appeared not to be affected by either age or diet. On several occasions, 50 lb. of nonfat milk was sham-fed as ten successive 5-lb. increments, and the PGE activity of each increment was measured. The activity of successive increments, expressed as per cent of the first, was as follows for Groups I and II, respectively: 100, 51, 36, 31, 27, 23, 21, 19, 18, and 17; 100, 42, 28, 22, 19, 18, 16, 15, 13, and 11.

P31. Effects of nonfat milk solids and other compounds on the activity of pregastric esterase, liver esterase, and pancreatic lipase.* H. A. RAMSEY, North Carolina State College, Raleigh.

The effects of various concentrations of nonfat milk solids (NFMS), vitamin-free casein, egg albumen, lactose, and calcium ($CaCl_2$) on the in vitro activity of progastric esterase (PGE), liver esterase (LE), and pancreatic lipase (PL) from the calf were studied. The source of PGE was sham-fed water collected at the cardial end of the esophagus via a rumen fistula, whereas LE and PL were obtained from acetone powders of fresh tissue. Enzyme activity was determined at the pH optima, according to the procedure described in *Clin. Chem.*, **3**: 185, 1957, with 2-naphthyl laurate as the substrate. When measuring the activity of PL, sodium taurocholate was included in the substrate mixture. Concentrations of added compound, mg/ml of enzyme preparation, ranged upward to the following maxima: NFMS, 8.0; casein, 2.56; egg albumen, 2.56; lastose, 4.24; and calcium, 0.088. The controls contained no added compound. Substrate hydrolysis, expressed as per cent of the control, for the aforementioned maximum concentrations were as follows for PGE, LE and PL, respectively: NFMS, 201, 104, 110; casein, 125, 106, 107; egg albumin, 173, 110, 118; lactose, 108, 105, 105; and calcium, 109, 102, 54.

* This investigation was supported in part by a research grant, A-2230, from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

P32. Utilization of various carbohydrates by young calves. M. OKAMOTO, J. W. THOMAS, AND T. L. JOHNSON, Dairy Cattle Reseach Branch, USDA, Beltsville, Maryland.

The extent to which young calves utilize various carbohydrates was determined by estimating the changes that occur in blood sugar concentrations after the carbohydrates were consumed by fasted calves. Glucose, lactose, sucrose, and dextrin were given at the rate of 200 g/cwt in aqueous solution or suspension. They were given to eight calves when 1, 2, 3, and 4 wk. of age. Another calf was given only water. At 5 and 6 wk. of age, the calves were given starch, dextrin, or sucrose, or these carbohydrates plus enzymes. After glucose or lactose administration, the blood sugar levels increased markedly. Maximum levels were attained between 1 and 2 hr. and after 5 hr. the levels were still from two to three times higher than the fasting levels of 60 to 80 mg.%. Sucrose, dextrin, starch, or water produced essentially no changes in blood sugar levels during a 5-hr. period (maximum increase of 0 to 11 mg.%). Dextrin gave a slightly greater response at 5-6 wk. of age than at 1-4 wk. Sucrose plus invertase produced blood sugar changes similar to glucose feeding. Fungal amylase fed with dextrin or starch did not increase the response. Pancreatic diastase (4%) fed with dextrin increased the response slightly. The response to lactose was larger at 3 and 4 wk. of age than at 1 and 2 wk. of age in each of three calves. Young calves up to 6 wk. of age were found to be able to utilize glucose and lactose, but were unable to hydrolyze sucrose, dextrin, or starch.

P33. Utilization of various sugars by the young dairy calf. J. G. VELU, K. E. GARDNER, AND K. A. KENDALL, University of Illinois, Urbana.

Twenty-one young fasted calves of various ages were tested to study their capacity to utilize several chemically pure sugars. The levels of blood-reducing sugars determined at regular intervals over a period of 4 hr. following the oral administration of the sugars were used as the measure of availability of each sugar tested. Sugars were fed in water to calves following a 24-hr. fast at the rate of 2 g. per pound body weight. Prior to treatment, blood sugars averaged 95, 73, and 62 mg. at 1, 2, and 3 wk., respectively, and near 60 mg. thereafter. Glucose feeding produced the highest blood sugar levels: 225, 224, 272, 286, 361, 385 mg. during the first 6 wk. Lactose and galactose gave slightly better levels at 1 wk., but somewhat lower than glucose thereafter. No scours resulted in glucose, galactose, or lactose administration. Calves showed no utilization of sucrose and poor absorption of fructose. Calves used maltose very poorly at 2 wk., but slightly better at 3 wk. Calves scoured on sucrose, fructose, and maltose. Sucrose inverted by invertase, or by citric acid, or fed with invertase showed high utilization.

P34. Effect of administering several absorbable chemical compounds on the development of rumen papillae in the young dairy calf. E. G. SANDER, R. G. WARNER, AND J. K. LOOSLI, Cornell University, Ithaca, New York.

Twelve Holstein calves equipped with rumen cannulae were fed milk and housed on elevated wooden platforms to prevent bedding consumption. Similar amounts by weight of five compounds: (1) sodium butyrate, (2) sodium propionate, (3) sodium acetate, (4) glucose, and (5) sodium chloride, were administered through the fistula over an 11-wk. period. Two calves serving as controls received (6) milk alone. A rumen papillary rating from 1 through 7 was given to each of the calves, with a score of 1 denoting poor, and 7 excellent, papillary development. The scores assigned to each of the treatments were, respectively, (1) 6, (2) 5, (3) 3, (4) 3, (5) 2, and (6) 1, indicating that butyrate and propionate stimulated more papillary growth than did the other treatments. Since the rates of absorption of the three principal volatile fatty acids from the rumen appear to be equal on a molar basis, it would seem that some phenomena other than absorption per se stimulates papillary growth. It is postulated that the degree of metabolism of the fatty acids by the rumen mucosa may be an important factor in papillary growth. These observations suggest that sodium chloride or glucose may also stimulate papillary development.

P35. Effect of various substances on the anatomical development of the rumen in the calf. H. TAMATE, A. DARE MCGILLIARD, R. GETTY, AND N. L. JACOBSON, Iowa State College, Ames.

Four-day-old calves were allotted to the following diets: I, milk; II, milk, hay, and grain; III, milk + acetic-propionic acid; IV, same as III + plastic sponges; V, milk + butyric acid; VI, milk + carbonated water; VII, milk + NaCl and KCl; VIII, milk + $NaHCO_3$ and $KHCO_3$. Milk was given by nipple, aqueous solutions of salts and acids by stomach tube into the rumen, and sponges by capsule. All calves, except on Diet II, were kept on screens. Two calves on Diet I and two on Diet II were sacrificed at each of the following ages: birth, 2, 4, 8, and 12 wk.; one calf was sacrificed at 8 wk. on each of the other diets. Calves on Diet II showed typical rumen development; those on Diet I showed no rumen development. Increase in length of rumen papillae coincided with the decrease in numbers per $cm.^2$ on Diet II. Compared to Diet I, Diets III to VIII (except IV) generally did not increase reticulo-ruminal capacity, but decreased omaso-abomasal capacity. The calf on Diet IV showed marked rumen development (except for papillae). Little papillary development occurred in the rumens of calves on Diets III to VIII. Calves fed acids had fold-like structures in the ruminal mucosa, and atypical papillae. Calves fed acids began "ruminating" at about seven days of age.

P36. Influence of roughage quality upon rumen development in dairy calves. A. M. SMITH AND D. A. SOBREVILLA, Vermont Agricultural Experiment Station, Burlington.

Five pairs of male dairy calves were fed and managed the same, except one member of each pair received high-quality mixed hay harvested June 25, whereas its mate received hay from the same field harvested July 15. Colostrum milk was fed the first three days. Whole milk was then fed at 10% of the body weight daily for 5 wk. After this time, the entire energy intake of the calves was derived by ad libitum consumption of the prescribed chopped roughage. Calves were sacrificed by pairs at various ages up to 18 wk. Rumen development was determined by volume, weight, and papillae development. Complete intake and growth data will be presented. Most significant was the tremendous difference in papillae development observed between roughage treatments at all ages. Two calves were raised on whole milk for a base comparison. Continuous digestion data were determined on two pairs of calves. The calves receiving the good-quality forage reached a peak of digestive ability at 2–3 wk. equal to that of mature steers. The calves receiving the poor-quality forage failed to reach the expected digestive ability even at 18 wk. of age.

P37. Corn silage as the only roughage for dairy calves. W. R. MURLEY AND R. D. MOCHRIE, North Carolina Agricultural Experiment Station, Raleigh.

Four trials were conducted to determine the combination of silage ad libitum and restricted calf starter that would promote growth through 4 mo. of age equal to that of calves fed hay and starter. Sixty calves (12A, 12H, 12G, and 24J) were allowed limited whole milk to 35 days and one of three levels of maximum daily starter intake: 2.0, 3.0, and 4.0 lb. for G and J, and 2.5, 3.5, and 4.5 lb. for A and H. One of a block of four similar calves was fed alfalfa or oat hay and the middle level of starter; the other three, one on each starter level, were fed silage. Calves fed hay and those fed silage with low to high levels of starter had average feed intakes of: 177, 181, 166, and 167 lb. of milk; 261, 193, 271, and 331 lb. of starter; and 214, 395, 328, and 259 lb. (as-fed) of roughage. Average daily gains were: 1.13, 0.87, 1.06, and 1.17 lb., with the difference between the hay and the silage calves on the low level of starter being at $P < 0.01$, as was a like difference between these two groups for increase in height (three trials). It appears that calves fed corn silage up to 4 mo. of age make gains comparable to those fed good-quality hay and similar amounts of milk and starter.

P38. Raising of dairy calves with limited amounts of milk. R. D. CLARK AND F. W. WHITING, Canada Department of Agriculture, Lethbridge, Alberta.

Seventy Holstein heifer calves were used to determine (1) whether there was any difference in growth rate of heifer calves fed whole milk at a daily rate of 10% of body weight or at a constant daily amount of 8 lb. until 4 wk. old, (2) whether calves fed limited quantities of milk can be weaned abruptly from milk, (3) whether calves will make normal growth when fed 6 lb. whole milk daily until 4 wk. old and no milk thereafter, and (4) whether cud inoculation will increase body growth.

Calves fed whole milk at a constant rate of 8 lb. per head daily until 4 wk. old made as rapid growth to 16 and 52 wk. of age as those fed milk at a rate of 10% of body weight. Calves weaned from milk abruptly when 4 wk. old gained as well as calves receiving the same total quantity of milk but weaned gradually. Inoculation of calves with cuds at frequent intervals did not increase body gains.

Calves that received 6 lb. of whole milk daily until 4 wk. old and none thereafter gained as well as those that received 8 lb. of whole milk until 4 wk. old or those that received 6 lb. of whole milk daily until 8 wk. old.

P39. Sex chromatin in the nuclei of bovine somatic tissues. D. R. LANG AND W. HANSEL, Cornell University, Ithaca, New York.

The discovery in 1949 of a sexual dimorphism in interphase neural cells of cats was followed by reports of a similar dimorphism in other mammalian species and cell types. Somatic tissues of cattle, because of their high chromatin content, were purported to be unsuitable for describing the sex chromatin, although it was observed in neural cells of normal and freemartin calves. In the present studies, liver tissue samples from 16 cows, eight bulls, and six steers, were fixed in modified Davidson's solution, hydrolyzed in hydrochloric acid, and stained in Thionin solution. Observations were made on 100 cells from each sample. The sex chromatin was attached to the nuclear membrane and stained a metachromatic violet, whereas the nucleolus retained the orthochromatic blue. Seventy per cent of the female cells exhibited the sex chromatin. Male tissue characteristically displayed the chromatin in less than 10% of the cells examined.

P40. Inheritance of the β-lactoglobulins in cow's milk. R. D. PLOWMAN, R. E. TOWNSEND, C. A. KIDD, AND S. N. TIMASHEFF, Dairy Cattle Research Branch and Eastern Utilization Research and Development Division, USDA.

Two forms of β-lactoglobulin (A and B) have been found in the milk of individual cows. Previous workers have postulated that the formation of these proteins is genetically controlled. Paper electrophoresis was used to determine which β-lactoglobulins were present in the milk of 131 cows from the Holstein herd at Beltsville. The milk of 33 cows contained the A form only, 28 contained the B form only, and 70 contained both A and B. A study of 42 offspring, resulting from matings of parents for which the β-lactoglobulin types had been established, indicated that differences in this characteristic are determined by a single pair of alleles with no dominance. The gene frequency was 0.52 for the A gene and 0.48 for the B gene. The observed frequencies of the three genotypes agree with expected, assuming random mating with respect to this character ($P = .73$). The genotypes of eight bulls were inferred from daughter-dam information. Six of these bulls appear to be of Genotype AB and two appear to be AA. Limited data on five other bulls indicate that two of them could be AB or BB and that the other three could be AB or AA.

P41. Evidence for genetic influence determining the composition of milk. R. G. WASHBURN, L. O. GILMORE, AND J. M. TREECE, Ohio Agricultural Experiment Station, Wooster.

Aliquot composite milk samples on individual cows obtained semimonthly were analyzed for fat, protein, and total solids. Ten-month lactation totals for pounds of total milk, total solids, fat, protein, and protein per cent were calculated for first and second lactations of 35 cows sired by six bulls. These data were analyzed in an effort to determine if inherited differences influence the extent to which these traits are manifested.

No lactation differences were found for total solids, protein, or fat yields. Significant sire differences (P. 01) were found for total milk, total solids, protein yield, and protein per cent. A sire difference (P .05) was found for fat yield. The ranking of sire groups for total milk was the same as for total solids, but differed from the other traits.

Furthermore, with the exception of two brothers, the sires found in groups in which they were statistically indistinguishable from each other for a given trait were different in all other traits.

It is concluded that these data show an inherited difference in the transmitting ability of these sires that is measurable in these five production traits. There are similarities between related bulls that are assumed to be due to inheritance.

P42. Evidence of inherited influences affecting casein yield and percentage as measured by the relationships to sire groups and cellular antigens. J. M. TREECE, L. O. GILMORE, R. G. WASHBURN, AND N. S. FECHHEIMER, Ohio Agricultural Experiment Station, Wooster, and The Ohio State University, Columbus.

From 56 lactations of daughters which were sired mostly by six bulls, an attempt was made

to ascertain if inherited influences affected the casein yield and content in the milk. These two traits, together with protein yield and content, were studied for association with cellular antigens located on eight chromosomes. With respect to sire differences, it was found that casein percentage in milk was influenced by the sire ($P < .001$) and the lactation yield of casein was also significantly affected ($P < .06$). A total of 32 possible relationships between antigen loci and milk traits were studied by analysis of variance. In both protein and casein percentages, there was a highly significant difference ($P < 0.1$) between the cattle possessing antigen L as compared to those not possessing this antigen. These differences were of the magnitude of .14–.17 percentage units in favor of the cows not possessing the antigen, and the actual difference was essentially the same for both traits. This similar difference suggests that the influence may be entirely on the casein value. In terms of percentage, the difference was approximately 5.5 for protein content and 8 for casein content.

P43. Relationship between certain factors and maximum daily milk production. J. M. RAKES, O. T. STALLCUP, O. H. HORTON, AND W. GIFFORD, University of Arkansas, Fayetteville.

Milk production records analyzed in this study were collected over a 40-yr. period. Approximately 66% of the cows studied remained in the herd through the second lactation. The older cows required longer to reach peak production in both the Holstein (38.3 days) and Jersey (42.7 days) breeds. Mean production peaks tended to be higher after the third lactation, with a maximum for the Jerseys of 36.4 lb/day and for Holsteins 64.4 lb/day. The average increase of the peak production in subsequent lactations over first lactation ranged from 15.4% in the second lactation to 47.9% in the eighth for Jerseys and from 18.6 to 92.0% in respective lactations for the Holsteins.

The correlations between peak production and total lactation yield were highly significant, having values of 0.67 and 0.72 for the Jersey and Holstein breeds, respectively. Correlation between time required to reach maximum daily production and production at the peak was 0.83 and 0.17, respectively, for the Holstein and Jersey breeds. Heritability estimates of peak production obtained for the Jersey and Holstein herds were 0.47 and 0.10, respectively.

P44. Some hereditary and environmental aspects of persistency of milk yield of Holstein-Friesians in Louisiana. CECIL BRANTON AND G. D. MILLER, Louisiana State University, Baton Rouge.

Persistency indices for 220 lactations of 127 Holstein-Friesian cows that calved during 1955, 1956, and 1957 in the Louisiana State University herd were computed and related to season and year of calving. These cows were daughters of 30 bulls.

The maximum daily and monthly milk yields were obtained in the second month of lactation for the majority of the cows, regardless of season and year of calving. Season and year of calving were found to have a highly significant effect on persistency of production. Cows calving in August–November had the highest persistency, those calving December–February were intermediate, and those calving March–June were the least persistent. Heritability and repeatabiltiy of persistency of production also are being estimated from these data.

P45. Associations of postpartum intervals with body weight loss, milk production, and butterfat percentage to 90 days following calving. S. E. MARES, A. C. MENGE, AND W. J. TYLER, University of Wisconsin, Madison.

Data from 356 service periods of 208 Holstein cows have been analyzed to relate various postpartum reproductive intervals with weight loss, milk production (M.E.), and butterfat percentage to 90 days postpartum. These animals included six sire-lines and two systems of mating (outbreds and inbreds, with inbreeding coefficients not exceeding 28%). Service periods in which abnormal calvings or cystic ovaries occurred have been omitted. After adjusting for parity and season at calving, correlations and regressions were calculated between cows within sire-lines and systems of mating. The average intervals from calving to first estrus, first corpus luteum, and involution of the uterus were 32.9, 19.2, and 43.6 days, respectively. The average milk production (M.E.), butterfat percentage, and weight loss for the first 90 days following calving were 4,856 lb., 3.48%, and 24 lb., respectively. Correlations of 0.16 ($P < 0.05$) existed for weight loss with the interval both to first estrus and to first corpus luteum. The correlation of milk production with weight loss was 0.18 ($P < 0.05$), with interval to involution 0.28 ($P < 0.01$), and with interval to first estrus 0.29 ($P < 0.05$) for inbreds and 0.09 for outbreds. Butterfat percentage had a correlation of 0.20 ($P < 0.01$) with weight loss, but had no significant association with the postpartum intervals studied.

P46. Relationship between milk production, body weight, and average daily gains to 6 mo. of age. E. W. HOLTZ AND R. E. ERB, State College of Washington, Pullman.

Two hundred experiment station cows of three breeds in two separate herds (Guernseys, Holsteins, and Jerseys) were used to determine if average body weight (pounds) at mid-lactation and average daily gain (pounds) from birth to 6 mo. of age (gain) was related to later yield of 4% fat-corrected milk (FCM). The Holsteins were in two herds. Feeding and management of the calves and cows was above average. Linear regression and correlation coefficients were determined by covariance. Cor-

relations and regressions on a cow-within breed basis were respectively: a) Gain $(g) \times$ weight $(w) = 0.32$ and 178 lb. b *w.g.*; b) gain $(g) \times$ FCM $(y) = +0.02$ and 178.0 lb. b *y.g.*;c) weight $(w) \times$ FCM $(y) = 0.39$ and 6.64 lb. b *y.w*; d) gain $(g) \times$ FCM/1,000 lb. of cow $(z) = -.16$ and $-1,225$ lb. b *z.g.*; e) gain $(g) \times FCM^{0.75} (m) = -0.12$ and 963 lb. b *m.g*; and f) weight $(w) \times FCM/W^{0.75} (m) = +.02$ and $+0.3$ lb. b *m.w*. If one can assume that $W^{0.75}$ correctly estimates metabolic capacity then, in this study, differences in body weight essentially account for differences in yield.

P47. Effect of age at puberty, 6-mo. weight, and calving weight on 90-day production in the first lactation of Holstein heifers. A. C. Menge, S. E. Mares, W. J. Tyler, and L. E. Casida, University of Wisconsin, Madison.

Associations of milk production and butterfat percentage for the first 90 days of the first lactation with 6-mo. weight, age at puberty, and calving weight (obtained three–seven days postpartum) were calculated for 141 Holstein heifers from six sire-lines. Six-month weight was significantly correlated with calving weight (.50) and milk production (.35). The associations of 6-mo. weight with fat percentage were heterogeneous among the sire-lines. Age at puberty was significantly correlated with 6-mo. weight (−.56), calving weight (−.31), milk production (−.18), and fat percentage (−.31). Milk production and fat percentage were significantly correlated with calving weight (.48 and .28, respectively). Path coefficients indicated that 6-mo. weight affected milk production to a greater extent through calving weight than directly or through age at puberty; whereas, it affected fat percentage to a greater extent through age at puberty than directly or through calving weight. Age at puberty had a negligible effect on milk production when calving weight was held constant, but calving weight had an effect when puberal age was constant. Both age at puberty and calving weight affected the fat percentage significantly, each independently of the other.

P48. Variables affecting the ratio of total milk produced in 305 days to monthly milk production. R. C. Lamb and L. D. McGilliard, Michigan State University, East Lansing.

Milk production on test day for 16,272 lactations recorded by Michigan DHIA-IBM from 1953 through July, 1957, was used to study variables affecting the ratio of total milk produced in 305 days to production in each month, and to develop factors for extending parts of lactations to 305 days.

Variance components for ratios of total to monthly production were used to determine for each breed the relative effect of season of freshening, lactation number, and age of cow at freshening. The components of variance were largest for season of freshening and lactation number. There was no significant difference between second and later lactations; hence, these were treated as a single group. There was no difference between herds, but a definite difference between breeds, with the low-fat breeds tending to group separately from the high-fat breeds.

Factors to extend part-time records to a 305-day basis were developed from the ratios of total to part production. Separate factors were developed for Holsteins, Guernseys, Jerseys, and Brown Swiss, with factors within breeds for all combinations of lactation number (first and later) and season of freshening (barn season and pasture season).

P49. Some effects of inbreeding with selection on milk yield and growth in milk goats. E. E. Ormiston and R. W. Touchberry, University of Illinois, Urbana.

Curvilinear and linear regressions of yield and growth on the coefficient of inbreeding were determined from milk and butterfat records of 52 French Alpine does, and monthly measures of wither height and body weight on 42 female kids. Milk yield was the criterion for selection.

The production data were analyzed within-sires and over-all, with and without the records adjusted for environmental differences, and within years. Regressions of the growth data on inbreeding were determined within-years, within-sires, and over-all. The curvilinear regressions of yield on the coefficient of inbreeding approached significance or were significant at the 5% level of probability. The linear regressions of yield on inbreeding were predominantly positive, but not significant. The effects of inbreeding on growth were slight. Curvilinearity of regressions of wither height at 2 and 10 mo. and of body weight at 10 mo. on inbreeding were not significant. Of the linear regressions of growth factors on inbreeding, only wither height at 10 mo. was significant, and it was positive.

P50. Effects of crossbreeding on milk and butterfat yield. R. W. Touchberry and F. N. Dickinson, University of Illinois, Urbana.

This study involved cattle of the Holstein and Guernsey breeds and crosses between these two breeds. The average relationships among the foundation females of the same breed and between a sire and the females of the same breed did not exceed 0.04. With these very low coefficients of relationship, the results of this study are measures of the general combining ability of these two breeds.

The mating system was such that contemporary purebreds of both breeds and reciprocal crossbreds were produced each year. Furthermore, the crossbreds contained essentially the same genes as the purebreds. The averages of the first lactation records of 38 purebreds and 42 crossbreds were 9,177 and 9,587 lb. of milk and 359 and 411 lb. of fat, respectively. It is apparent that crossbreeding has resulted in

4.5 and 14.6% increases in first-lactation milk and fat production, respectively.

P51. Effect of age, season, stage of lactation, classifier, and year on type ratings of Holstein cows. C. J. Wilcox, K. O. Pfau, R. E. Mather, and J. W. Bartlett, New Jersey Agricultural Experiment Station, Sussex.

Fall and spring classification ratings by 13 official inspectors from 1946 to 1957 were coded to give unit intervals (1.0) between ratings. Ages (lactation numbers) were 1, 2, 3, 4, 5, or 6+. Lactations were divided into three stages, (a) parturition to 60 days, (b) 61 to 240 days, and (c) 241 days to next parturition. Cows with single classifications were excluded; 2,272 classifications of 223 cows remained. To remove the confounding of each variable with each other, least squares analyses with dummy variables were utilized. Estimates of environmental effects on a total, among-cow, and within-cow basis were obtained through use of an electronic computer (IBM 650). On a total basis, coded over-all ratings increased steadily with age with a range of 1.04 between lactation 1 and 6+; spring ratings were higher than fall by 0.06 ($P < 0.10$); early- and late-lactation scores were higher (0.12* and 0.18**) than mid-lactation; the range among classifiers was 1.29. Trends among subratings were similar, except that feet and legs, and fore udder, showed smaller age and greater classifier effects; dairy character decreased (0.15**) and body capacity increased (0.31**), in late-lactation. Linear year effects were detected of −0.03** for over-all rating, with a range of +0.03* to −0.05** for the subratings.

P52. Comparison of the type characteristics of naturally and artificially sired Holstein-Friesian cows in Ontario. J. C. Rennie and G. R. Barr, Ontario Agricultural College, Guelph, Canada.

This study, based on type records of 69,124 Holstein-Friesian cows officially classified in Ontario from 1953 to 1957, inclusive, was undertaken to evaluate what genetic advantage had been realized in terms of type by the use of artificial insemination in Ontario. Of the cows classified during the period under study, 46.6% were artificially sired, with 4.8% of these being in herds where artificial insemination was the only breeding system used. With the official type ratings being Excellent, Very Good, Good Plus, Good, Fair, and Poor, the artificially sired (A.I.) cows ranged from 46.8% to 48.3% Good Plus and better on final rating in 1953 and 1957, respectively, compared to 48.7% and 48.2% for the naturally sired (N.S.) cows in the same years. The average score of the (A.I.) cows rose from 78.2 in 1953 to 78.8 in 1957, whereas that of the (N.S.) cows rose from 78.6 to 78.9 in the same period, indicating a greater rate of increase in score for the (A.I.) cows than the (N.S.) cows. Considering the eight sections of the score-card breakdown over all years, (A.I.) cows equalled or excelled (N.S.) cows in udder characteristics, but were lower in the other sections, particularly feet and legs.

P53. Heritabilities of type appraisal traits and their genetic correlations with production. L. D. Van Vleck, G. V. O'Blenes, and C. R. Henderson, Cornell University, Ithaca, New York.

Heritabilities and genetic correlations were estimated by daughter-dam comparisons on a within herd-year basis from New York dairy type appraisal records for Holstein-Friesian cows for which 2 × M.E., 305-day records were available. Heritabilities for production and 27 type appraisal traits and genetic correlations for production with these traits were obtained from 842 pairs in 178 herds. Some of the heritability estimates are as follows: temperament, .36; udder texture and strength of rear udder attachment, .23; depth of udder, .21; pin-bone width, udder shape, and hind legs, .04. Some of the estimated genetic correlations of type traits with milk production are as follows: dairy character, .95; feeding habits, 1.04; withers, .22; temperament, .05; and rear leg movement, −.10.

P54. Heritability and repeatability estimates of production and type of Guernsey cattle. E. R. Berousek, J. A. Whatley, Jr., R. D. Morrison, S. D. Musgrave, and W. R. Harvey, Oklahoma State University, Stillwater, and Agricultural Research Service, Beltsville, Maryland.

Knowledge of the heritability and repeatability of milk, butterfat, and type is important in evaluating the effectiveness of a selection program. The production records and official type ratings of 3,202 Guernsey cows, including 1,981 daughter-dam pairs located in 239 herds, were involved. Production records were adjusted to a 2 ×, 305-day, M.E. basis. Type ratings included nine breakdown classifications and a final rating. The intraherd, intrasire regressions and correlations of daughter on dam were used in estimating heritabilities, and the repeatability estimates were computed from the within- and between-cow analysis of variance.

On a single record basis, the heritability of milk and fat yield was 0.18 and 0.19, respectively. Correspondingly, the repeatability estimates for milk and fat were 0.47 and 0.41.

The heritability of final type rating was 0.28 and the repeatability estimate was 0.43. The heritabilities of the breakdown classification ratings ranged from 0.16 for dairy character to 0.32 for teat placement. Repeatability estimates of these same ratings ranged from 0.20 for dairy character to 0.52 for rump. The heritabilities of these ratings were most frequently between 0.22 and 0.28, whereas most of the repeatabilities ranged from 0.33 to 0.44.

P55. Expected genetic progress by progeny testing. C. R. HENDERSON, Cornell University, Ithaca, New York.

The expected genetic progress resulting from one cycle of progeny testing is a complicated function of heritability, number of sires tested, number of progeny per tested sire, and number of sires selected. Fast computers make it possible to calculate the expected progress for many combinations of these variables. This has been done for heritability = .25, using an IBM 650 computer. The resulting values have been examined for suitably accurate approximations that are simple to compute. Two of the more interesting results of this investigation are:

1. The optimum ratio of selected to tested sires is almost a linear function of the square root of the total number of tested daughters in the program.

2. The expected genetic progress using the optimum ratio of selected to tested sires is approximately a linear function of the 0.2 power of the total number of tested daughters.

These two results emphasize the importance of testing a large number of sires per sire selected and of conducting progeny testing on a large scale.

P56. Isotope dilution as a measure of acetate "pool" size, turnover time, and endogenous acetate production. C. L. DAVIS, R. E. BROWN, J. R. STAUBUS, AND W. O. NELSON, University of Illinois, Urbana.

A 350-lb. fistulated steer was used to obtain preliminary data on the use of acetate-1-C^{14} to measure the size (weight), volume, and the turnover time of the acetate "pool," and to ascertain the magnitude of endogenous acetate production. The exogenous sources of acetate were minimized by starving the steer 72 hr. and emptying the rumen 15 hr. prior to the intravenous injection of acetate-1-C^{14}. Venous samples were obtained at various times up to 6 hr. The specific activity of blood acetate dropped rapidly during the first 30 min. and approached a steady state at 60 min. after injection. The rapid drop in specific activity immediately after injection is attributed to dilution by acetate in the pool and to rapid metabolism of the labeled acetate before mixing is complete. The latter loss is considerable (approximately 95%); hence, the relation of total injected counts to the steady state specific activity versus time curve extrapolated to zero time gives an erroneously high value for pool size. Calculation of the turnover time from the steady state specific activity-time curve gave a value of 1 hr. The concentration of acetate in the blood remained relatively constant at 0.6 mg. % during the experiment.

P57. Water consumption in dairy cattle as influenced by environmental temperatures and urine excretion.* TAYMOUR H. KAMAL, HAROLD D. JOHNSON, AND A. C. RAGSDALE, University of Missouri, Columbia.

The fact that water consumption and, consequently, milk production are closely related to environmental temperature has prompted this experiment to show whether water intake in cattle is controlled by environmental temperature directly or through urine excretion. Nine Brown Swiss, Holstein, and Jersey heifers were exposed to six temperature levels (35 to 95° F.). As temperature increased from 35 to 95°, water consumption increased from 22.8 to 67.0 liters per day and urine excretion increased from 3.8 to 24.8 liters per day. Thereafter, each of two Jersey heifers was injected with 20 pressor units of antidiuretic hormone at 68 and 92° F. Following injection of antidiuretic hormone, water intake was blocked in Jersey 633 and Jersey 635 for 4.5 hr. and 10 hr., respectively. Meanwhile, the urine excretion gradually depressed 90% at both temperature levels. This indicates that environmental temperature apparently influences water intake not directly, i.e., via "drinking center," but probably through urine excretion. Our previous data suggest that the mineral-corticoids' activity is depressed at high temperature, a fact that may contribute to increase of urine excretion and, probably, consequent effect on blood osmotic pressure, "osmo receptors," and water intake.

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P58. Calcium and inorganic phosphorus levels in bovine blood serum and urine, following dihydrotachysterol administration. K. A. KENDALL AND K. E. HARSHBARGER, University of Illinois, Urbana.

Seven nonlactating dairy cows were used in which two were maintained as controls and five were treated with 15 to 25 mg. of dihydrotachysterol. Blood and urine samples were taken 3 and 0 hr. before treatment, and at 3, 6, and 9 hr. after treatment. Blood serum calcium levels rose from 10.9 to 11.5 mg. % 6 hr. after treatment, whereas inorganic phosphorus values increased from 3.8 to 4.2 mg. % during the same period. Urinary calcium concentrations steadily rose from 3.35 to 5.49 mg. % during the 9-hr. period following treatment. Urinary inorganic phosphorus levels for the same period showed little change. Average urinary volumes for the 0-3, 3-6, and 6-9 hr. periods following treatment were 1,255, 1,333, and 1,460 ml., respectively.

P59. Twenty-four-hour 17-keto steroid excretion in the cow. W. F. WILLIAMS, University of Maryland, College Park.

The daily excretion of 17-keto steroids by the cow has been determined from composite 24-hr. urine-feces collections. Δ^4-Androsten-3,

17-dione-4-C^{14} has been added in known amounts to correct for steroid loss during extraction.

The aliquot for extraction is oven-dried, extracted with benzene: ethyl alcohol, the benzene: ethyl alcohol distilled off, and the residue taken up in 70% ethyl alcohol. The alcoholic solution is partitioned against hexane, taken to dryness, and dissolved in ether. The ether solution is treated with sodium hydroxide, followed by water washes to give a "neutral extract." The neutral extract is treated with a Girard separation for ketones and the ketonic material taken to dryness.

The ketonic material in carbon tetrachloride is chromatographed on alumina with small per cents of ethyl alcohol, and the recovered steroids and pigments are rechromatographed on alumina with carbon tetrachloride: ether mixtures. The steroids with minor amounts of pigment are then quantitatively determined, using the antimony trichloride reaction and subtracting a blank for pigment. After correction for extraction loss, the total 17-keto steroid excretion is found to be of the order of 500 mg. per day.

P60. Some blood changes following bilateral adrenalectomy in the bull calf. V. L. Estergreen, Jr. and N. L. VanDemark, University of Illinois, Urbana.

A Holstein bull calf was bilaterally adrenalectomized in two stages, at 23 and 52 days of age. Following removal of the second adrenal, the calf was kept in a heated room and substitution therapy was instituted. For this 110-lb. calf, 5 mg. DOCA and 25 mg. cortisone acetate or 5 mg. prednisone given IM every other day was found to be adequate to prevent onset of deficiency symptoms. Progressive weakness in the legs, lethargy, followed by loss of appetite and extreme apathy, were considered to be outward signs of adrenal deficiency. Cessation of steroid therapy caused deficiency symptoms to appear after the third day. This could be prevented by free-choice feeding of salt, but the deficiency symptoms would appear the fourth day after cessation of salt feeding. Depression of serum Na and elevation of serum K and eosinophil levels were clearly evident in the blood taken from the deficient calf. On the day preceding death, the serum Na, K, and blood eosinophil levels were 274 mg. %, 34.7 mg. %, and 9%, respectively, compared with mean levels of 313 mg. %, 21.9 mg. %, and 3% while receiving steroid and salt therapy. No consistent change in the other formed elements of the blood could be directly associated with adrenal steroid deficiency.

P61. Comparison of methods of measuring thyroid function in dairy cattle. J. E. Johnston, G. A. Hindery, W. T. Burnett, and A. Guidry, Louisiana State University, Baton Rouge.

Eight dairy heifers (four Jersey and four Holstein) were placed in a climatic control chamber under uniform light, temperature, humidity, and nutritional conditions for a 70-day period. The last 35 days of this period were utilized for the determination of thyroid function. Rate of destruction of thyroxine and biological half-life were determined from the rate of disappearance of I^{131}-tagged thyroxine from the blood. Hourly slope of the destruction curve ($-b \times 10^{-4}$) ranged from 45.1 to 70.8, with a mean of 55.1, whereas biological half-life ranged from 46 to 67, with a mean of 55 hr. PBI ranged from 5.0 to 9.1, with a mean of 7.7 mg.%. Correlation between the rate constant and PBI was −0.11. Thyroxine secretion rate was determined, using the thyroidal I^{131} release technique and values per 100 lb. of body weight ranged from 0.20 to 0.33, with a mean of 0.25 mg. per day. Correlation between thyroxine secretion rate and PBI was −0.03.

Metabolic rate was estimated from rate of oxygen consumption. These values had a correlation of 0.42 with rate of thyroxine destruction and of 0.90 with thyroxine secretion rate. This study indicates that PBI is a questionable index of thyroid function and that, of the various measures used, thyroxine secretion rate is most closely related to metabolic rate.

P62. Efficiency of absorption of crystalline L-thyroxine and of thyroxine in thyroglobulin from the gastrointestinal tract of lactating cows. J. P. Mixner and H. D. Lennon, Jr., New Jersey Agricultural Experiment Station, Sussex.

Five lactating cows (four Holsteins and one Guernsey) were used to determine the efficiency with which crystalline L-thyroxine and thyroxine in porcine thyroglobulin were absorbed from the gastrointestinal tract. The criterion of the absorption efficiency was the comparative elevation in the levels of plasma protein-bound iodine by the orally administered thyroid materials, as compared to intravenously injected L-thyroxine on a within-cow basis (*J. Dairy Sci.*, **41**: 728. 1958). Highly significant differences ($P < 0.01$) were found among animals with respect to their absorption efficiency. The mean absorption efficiencies of the crystalline L-thyroxine and the thyroxine from thyroglobulin were 10.85% (range of 4.2 to 17.2) and 10.20% (range of 4.2 to 16.5), respectively. The thyroxine content of the thyroglobulin (0.7%) was taken as the mean according to its biological activity (1%) and its Blau-I thyroxine (0.4%). The standard error of a single determination of thyroxine absorption efficiency was 0.95%, based on the cow times thyroxine-source interaction mean square, with a corresponding coefficient of variation of 9.03%. The efficiencies of absorption of thyroxine as determined here correspond well with those previously reported for thyroxine in thyroprotein (12.3%).

P63. Effect of feeding L-thyroxine upon plasma protein-bound iodine in lactating cows.* E. W. SWANSON AND A. F. MCFEE, University of Tennessee, Knoxville.

Plasma PBI determinations were made in three experiments, from 2 to 4 wk. before to 10 wk. after feeding L-thyroxine for 12 to 16 wk. to 28 cows in comparison with ten control cows. Peak PBI levels (10 to 14 γ%) were usually reached after 2 to 4 wk. of treatment. When thyroxine was fed for 14 wk. during the winter and spring, elevated PBI levels were maintained constantly for the full period. Treatments during the summer and fall resulted in a gradual decline from the peak values. The decline occurred even when daily thyroxine doses were increased in summer. After treatment ceased, PBI levels fell about 0.5 γ% below normal for 1 wk., but had returned to normal levels by the second week. PBI of thyroxine-treated cows remained normal through a 10-wk. post-treatment observation period. PBI levels were also normal in the succeeding lactations of three sets of identical twins used in long term thyroxine vs. normal feeding comparisons. When daily doses of 60 to 160 mg. of L-thyroxine were fed, the effects of dose on plasma PBI were not directly proportional, as high dose levels were less efficiently transferred to plasma PBI than were low levels.

* Supported in part by a grant from Baxter Laboratories, Morton Grove, Illinois.

P64. Effect of sudden changes in forage ration on thyroidal I^{131} release rate. J. H. VANDERSALL, J. W. HIBBS, AND H. R. CONRAD, Ohio Agricultural Experiment Station, Wooster.

Previous reports showed that the sharp decline in milk production following certain forage ration changes could not always be explained on the basis of reduced feed intake and/or digestibility. Earlier work, based on thyroidal I^{131} uptake, indicated that the thyroid was nivolved. I^{131} release rates were measured in ten heifers fed alfalfa hay. The ration was then changed abruptly to legume-grass silage. The release rates, measured beginning nine days after the ration change, were increased ($P < 0.01$). Ten other heifers were used in the same manner to compare mixed hay (clover, grass, alfalfa), predominantly grass silage, and predominantly legume silage. The release rates of the heifers fed mixed hay and those fed legume silage were greater ($P < 0.05$) than those fed grass silage. In another experiment, the release rates increased ($P < 0.01$) in two of five calves changed from alfalfa hay to legume-grass silage seven days after the injection of the I^{131}. Increases in release rate were not observed when the same forage change was made using calves in which iodine reutilization was blocked by feeding thiouracil, or in control calves fed alfalfa hay throughout. These data indicate the presence of goitrogenic substances in some forages commonly fed to dairy cattle.

P65. Dry matter losses in small-stack silos as affected by covering techniques. J. C. DERBYSHIRE, C. H. GORDON, AND J. R. MCCALMONT, Dairy Cattle and Agricultural Engineering Research Branches, USDA, Beltsville, Maryland.

Various types of covers were applied to pilot stacks of corn and orchardgrass silage to determine their effectiveness in reducing dry matter losses. The effiectiveness of the covers in preventing spoilage was quite variable and no consistent effect was observed in these small stacks. On the other hand, all coverings, except a simple roof, improved dry matter recovery, thus indicating that the coverings had reduced gaseous and/or seepage losses. The relationship between spoilage and the gaseous and seepage losses also was quite variable. Some treatments appearing worthless as judged by amount of spoilage, materially reduced the gaseous and seepage losses. These observations emphasize that the effectiveness of silo-sealing techniques can not be adequately assessed by determination of top spoilage losses only.

P66. Some observations on stack silos sealed with plastic covers. D. L. HILL, C. H. NOLLER, C. M. BROWN, AND N. S. LUNDQUIST, Purdue University, Lafayette, Indiana.

During 1957 and 1958, stack silos sealed with polyethylene plastic tarps were observed under farm conditions. Ninety-eight silos ranging in capacity from 20 to 150 tons each were made. Green crops which were ensiled included alfalfa, alfalfa-grass mixtures, oats, wheat, barley, rye, corn, and sorghum. The amount of silage made per farm ranged from about 20 to 1,400 tons. The success of individual silos ranged from highly satisfactory to unsatisfactory. When good ensiling procedures were used, the silo properly and quickly sealed and the plastic not damaged, there was no surface spoilage, and excellent ensiling fermentations were obtained. Any loss after opening depended on the time of year and the time required to feed it. Less than highly satisfactory results were associated with poor ensiling procedures, improper sealing, or damage to the plastic. Improper sealing and damage to the plastic resulted in surface losses ranging from 2 to 16 in. in depth. Humans and domestic animals were most frequently involved in causing damage to the plastic. Farmers using the technique for the second year showed improvement, and were more successful than those using the technique or making silage for the first time. The results obtained demonstrate that excellent silage can be made using this method when attention is given to proper ensiling procedures and to protecting the plastic.

P67. Preservation losses in bunker, concrete stave, and in glass-lined silos. H. H. VOELKER, South Dakota State College, Brookings.

This research was conducted to obtain losses and chemical changes in different types of silos. Alfalfa having 72% moisture, preserved with 10 lb. sodium metabisulfite per ton, showed a total weight loss of 32.5% when stored in a wood bunker silo covered with 4 mil black polyethylene. Alfalfa, 51.5% moisture, stored in a Harvestore silo, had losses averaging 1.7%. Oat silage losses were determined similarly for 2 yr., using upright concrete stave and wood bunker silos. All silos were covered with plastic. The first year total losses were 29.6% of 114 tons in the concrete stave silo and 37.1% of 100 tons in the bunker. The second year, oat silage cut at two stages of maturity had losses: upright, soft dough stage, 14.6%, and early milk stage, 34.4%; bunker, soft dough stage 30.9%, early milk stage, 33.8%. Chemical changes (complete analyses and carotene) between ensiling and feeding, especially ash values, were closely related to total losses. These studies indicate the differences which occurred in preservation losses, possibly due to air-tightness of silos, surface exposed while feeding, depth of stored silage (upright or bunker), type and moisture content of crops.

P68. Value of bacitracin as a preservative for grass silage on milk production. L. L. RUSOFF, C. P. BREIDENSTEIN, AND J. B. FRYE, JR., Louisiana Agricultural Experiment Station, Baton Rouge.

White Dutch clover was ensiled in four plastic silos (20 tons each) with the following preservatives: (I) zinc bacitracin, 10 g/ton; (II) molasses, 80 lb/ton; (III) sodium metabisulfite, 8 lb/ton, and (IV) control, no preservative. All silages were of good color and odor. The effect of the silages on milk production was tested with 12 lactating cows in a 63-day reversal trial. A seven-day preliminary period was followed by a 14-day experimental period. The silages were fed ad libitum. Concentrates were fed 1:3 on a 4% FCM basis, and 5 lb. of hay was fed daily to provide sufficient dry matter intake. The average pH value, average daily silage consumption, average FCM value, and efficiency of milk production (pounds of total dry matter consumed per pound of FCM) of the silages were as follows: for (I) bacitracin-treated silage, 4.29, 67 lb., 26 lb., and 0.96 lb.; (II) molasses-treated silage, 4.47, 73.9 lb., 26 lb., and 1.07 lb.; (III) bisulfite-treated silage, 4.51, 63.3 lb., 24.1 lb., and 1.03 lb., and (IV) control, 5.17, 86.2 lb., and 1.03 lb., respectively. A significant difference ($P < .05$) was obtained only among the values for silage consumption.

P69. Further comparisons of alfalfa hay and alfalfa silage for growing dairy heifers. J. W. THOMAS, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Alfalfa hay and wilted alfalfa silage were made simultaneously from the same field and fed to four groups of Holstein and Jersey heifers. All calves were fed the same diet at 5 mo. of age, after which Groups A and B were fed the hay while Groups C and D were fed the silage. Limited grain was fed to all heifers until 8 mo. of age. At 12 mo. of age, the forage fed to Groups B and C was reversed. All heifers were fed hay, corn silage, and concentrates from 19 to 24 mo. of age. Heifers fed hay had greater body weight gains (166 to 220%), dry matter intakes (134 to 140%), and efficiency of gains (124 to 150%) than did heifers fed silage. D.M. intake decreased 3 lb/day within five days in Group B, when changed from hay to silage at 1 yr. of age. Group C increased a similar amount during the first 20 days of hay feeding. All groups, except Jerseys in Group A, had a marked increase in growth rate after 19 mo. of age. When 24 mo. of age, Holstein heifers in Groups A, B, C, and D weighed 1,220, 1,060, 1,110, and 1,030 lb., respectively. The Jersey heifers in Groups A, B, C, and D weighed 690, 700, 690, and 570 lb., respectively. These trials confirm earlier observations that wilted alfalfa silage was less satisfactory than hay for growing dairy heifers, and extend the information to include both young and older heifers fed silage for only a 7-mo. period.

P70. Effect of supplements on utilization of corn silage, oat hay, and Coastal Bermuda hay rations. W. A. KING, J. LEE, H. J. WEBB, AND D. B. RODERICK, Clemson College, Clemson, South Carolina.

Digestion and balance trials were conducted on rations containing (a) phosphated-urea-molasses fed with corn silage and (b) molasses or alfalfa leaf meal with grass hays. Dairy heifers and ten collection periods were used. The first year, the phosphated-urea-molasses contained 3% phosphate feed solution (75% H_3PO_4) and 4% urea; the second year, 4% phosphate and 4% urea. With the phosphated-urea-molasses rations, 20 g. trace-mineralized salt was fed per heifer daily. The other rations included 20 g. salt and 20 g. dicalcium phosphate per heifer daily.

Increasing the phosphated-urea-molasses intake from 3 lb. per heifer daily to 6 lb. significantly increased the digestibility of the dry matter and TDN content of corn silage rations. All the nutrients were efficiently utilized and no great differences were found between the urea-molasses containing 3% and 4% phosphate. Both blackstrap molasses and alfalfa leaf meal significantly increased the digestibility of the dry matter, protein, and NFE and TDN content of oat hay rations. Supplements of cottonseed meal and cottonseed meal and molasses significantly increased the digestibility of the dry matter and TDN content of Coastal Bermuda grass hay rations. The nitrogen, calcium,

and phosphorus retentions were good in each of the grass hay rations.

P71. Comparative value of forage sorghums and corn as silages for lactating cows.* F. G. OWEN, J. R. KUIKEN, AND O. J. WEBSTER, University of Nebraska, Lincoln.

Forage sorghum hybrid, RS 303F; sorghum variety, Axtell; and corn were compared as silages for lactating dairy cows (RS 301F is male sterile and produced very little seed). Twelve Jersey cows were used in a double-reversal design with three 21-day periods. Grain was fed on a constant level based on pre-experimental production and silage was fed ad libitum as the only roughage. For the corn silage, Axtell and RS 303F, respectively, FCM production averaged: 31.7 lb., 29.6 lb., 29.2 lb.; total silage intake/day: 46.2 lb., 42.9 lb., 41.5 lb.; silage dry matter intake/day: 11.1 lb., 11.7 lb., 11.3 lb.; body weight gain/day: 0.7 lb., 0.7 lb., 0.6 lb. FCM production and silage intake were significantly higher for corn silage compared to the sorghums. Silage dry matter intake, change in body weight, and per cent butterfat in the milk were not found significantly different among treatments. Since RS 301F produced very few seed, it appeared that at least part of the nutrients normally stored in the seed of sorghums was deposited in the stalk and leaves of the sterile sorghum plant.

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P72. Interaction between spring pasture and grass silage when fed to dairy cows. J. K. MILLER, W. J. MILLER, AND J. L. CARMON, University of Georgia, Athens.

A study was made to determine if an interaction existed between silage and pasture when the silage was supplemented with a limited amount of grazing on succulent spring pasture. In a 30-day continuous feeding trial, three comparable groups of six lactating dairy cows each were fed: (1) grass silage ad libitum, (2) rye and rye grass pasture ad libitum, and (3) silage ad libitum, plus 1 hr. of grazing daily. Concentrates were fed according to milk production in a prior standardization period. Pasture intake was estimated by the chromogens–chromic oxide technique. Average silage and pasture TDN intakes of the cows in the combination group, which were 86.5% and 24.8% of the average intakes of the silage and pasture groups, respectively, totaled significantly more than expected. The combination group produced 12.5% more 4% FCM than was predicted from the productions of the other two groups and the reduction in silage consumption of the combination group. This statistically significant increase in milk production of the combination group was attributed to increased TDN intake, rather than to interaction in efficiency of utilization of the combined roughages. No interaction was found in the digestibility of dry matter.

P73. Comparison of the performance of dairy cattle on continuously grazed and strip-grazed pasture. A. M. SOMMER, H. H. OLSON, A. REED, AND H. F. BENSON, Southern Illinois University, Carbondale.

Two similar groups of six dairy cows of Holstein and Guernsey breeds were assigned to two 6.3-acre plots of an orchardgrass-legume pasture. One plot was grazed continuously and the other strip-grazed. Total milk production and TDN production for a 125-day grazing period under the two management systems were compared. One pound of grain was fed for each 6 lb. of 4% FCM produced daily.

The cows on the continuously grazed plot produced an average of 31.9 lb., and those on the strip-grazed plot, 31.2 lb. 4% FCM daily. The total body weight change was a loss of 58 lb. in the group on the strip-grazed plot and a gain of 113 lb. in the group on the continuously grazed plot. In addition to the milk produced, the strip-grazed plot yielded 6,765 lb. of air-dried hay from two cuttings.

P74. Effects of potassium nitrate on bovine semen production and various spermatozoan characteristics.* J. D. SIKES, University of Missouri, Columbia.

Field reports of poor reproduction and general toxicity in dairy cattle associated with the consumption of feeds containing relatively large amounts of nitrates indicated the need for investigations on the effect of "nitrate toxicity" on reproductive capacity in the bull. A partial semen depletion technique was used to assess the effect of potassium nitrate intake on spermatozoan motility, morphology, concentration, and % alive, as well as volume of semen produced by 2-yr.-old Holstein bulls. The technique involved collection of up to ten ejaculates per bull during a 6-hr. period on Days 0, 7, 9, 14, and 21. Individual bulls received 5, 7.5, 10, 15, or 20 g. of potassium nitrate per 100 lb. of body weight for periods of 7, 7, 7 or 14, 14, and 3 days, respectively. In each instance, nitrate administration (by stomach tube) began on the day following the initial series of semen collections. Blood samples were taken from each bull approximately 5 hr. after nitrate administration. Maximum conversions of hemoglobin to methemoglobin on the final day of nitrate intake were 0, 0, 5, 9.8, 65, and 64%, respectively. Animals showing as much as 50% conversion of hemoglobin to methemoglobin exhibited the common symptoms of nitrate poisoning; however, libido, semen production, and the various spermatozoan characteristics, when compared to those of control animals, did not appear to be adversely affected.

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P75. Effects of various cooling methods on spermatozoan livability.* A. F. McFee, University of Tennessee, Knoxville.

Cooling diluted bovine semen from 32 to 5° C. slowly (0.1° C. per min.) or by controlled variable rates gave no better livability than an uncontrolled natural rate in a 5° C. refrigerator. Rapid cooling (1.0° C. per min.) markedly reduced sperm livability. For the initial dilution, 32° C. was optimum, compared to 16, 21, 27, and 38° C. This was true when cooling was naturally variable to 5° C., as well as when cooling rate and time were held equal. Livability studies were made after different cooling rates from 5° C. to 0, −5, and −10° C. Livability was better after cooling at 0.2° C. per min. than 0.5° C. per min., and both these rates were superior to 1.0° C. per min. Samples cooled to 0° lived better than those cooled to −5°; the latter were superior to those cooled to −10° C. Trials in which semen was cooled and stored at various dilution rates up to 1:500 showed that either cooling or storing at high dilution rates reduced spermatozoan livability, but that cooling was more detrimental than storage at high dilutions. A statistically significant difference in motility loss in four days of storage was noted between storage dilutions. Samples cooled at 1:5 and stored at higher dilutions maintained better motility than those cooled at higher dilutions.

* Supported in part by a grant from the National Association of Artificial Breeders.

P76. Gas-pressure technique for the preservation of bovine spermatozoa. R. H. Kliewer and F. B. Wolberg, Oregon State College, Corvallis.

This study was originated to compare motility, storage life, and fertilizing capacity of bovine spermatozoa subjected to 20 p.s.i. of CO_2, N_2, or A (argon) gases for 60 min. at 20–22° C.

Six hundred and five semen samples from 38 collections of seven dairy bulls were standardized to 4×10^7 spermatozoa/ml and added to vials containing 3 ml. of egg yolk–citrate (1:3) extender. The YC extender was fortified with 1,000 I.U. of penicillin (potassium crystalline G) and 1,000 μg. of dihydrostreptomycin sulfate/ml.

Unsealed vials of extended semen were placed in a pressure chamber at 20–22° C. After the chamber was flushed and sealed, the extended semen was subjected to 20 p.s.i. of gas for 60 min. After treatment, the samples were stoppered and sealed with liquid paraffin at atmospheric pressure. All samples were stored at 4° C.

Progressive motility ratings were made on the day of collection, and Days 3, 7, 10, and 14. Highly significant differences ($P < 0.01$) in the motility of N_2 and A treated semen and the controls were observed. Comparison of CO_2 treated semen with the controls was significant ($P < 0.05$). Motility results indicate that semen preserved by the gas-pressure technique can be used satisfactorily through Day 7.

P77. Use of egg yolk in skimmilk-glycerol diluents to improve bovine sperm livability and simplify glycerolation. E. W. Wickersham and J. O. Almquist, The Pennsylvania State University, University Park.

Addition of egg yolk to heated skimmilk–10% glycerol (SG) diluents significantly improved sperm livability during 2 wk. of storage. Studies revealed a highly significant yolk-glycerol interaction favoring levels of 10-28% egg yolk and less than 7.5% glycerol. Further studies involved attempts to reduce the time presently required to add glycerol to SG diluents. Analysis of a factorial experiment (1,620 motility estimations), using skimmilk with 0, 10, and 20% egg yolk and 2.5, 5.0, and 10.0% glycerol, showed a very small but statistically significant 1.3 percentage units difference in livability in favor of adding the glycerol fraction stepwise (20, 30, and 50% at 10-min. intervals) at 5° C. rather than all at once. Livability was significantly higher in skimmilk–egg yolk-glycerol (SYG) diluents containing 20:2.5, 10:2.5, and 20:5.0 (%yolk:%glycerol) than in all other combinations. Based on these results, various SYG combinations are being tested to determine whether the glycerolation procedure can be eliminated. Preliminary fertility trials indicate that SYG diluents are equally as good as milk-glycerol diluents.

P78. Coconut milk citrate plus egg yolk as an extender for bovine sperm at low temperatures. C. Norman, H. S. Min, I. D. Porterfield, and R. S. Dunbar, Jr., West Virginia University, Morgantown.

A comparative study of the motility of bovine sperm extended in skimmilk, egg yolk citrate, coconut milk citrate, and in various combinations of coconut milk citrate and egg yolk was carried out at 5 and at −79° C. Estimates of motility were made at zero time, immediately after lowering the sperm suspensions to the desired temperatures, and after exposure to low temperatures for one-, five-, and ten-day intervals. Analysis of the data demonstrated that a combination of 15% coconut milk and 5% egg yolk in a 2.16% solution of sodium citrate containing a final concentration of 10% glycerol was distinctly superior to skimmilk and egg yolk extenders at 5° C. The presence of the 5% egg yolk in coconut milk citrate effectively enhanced the motility and survival rate of sperm at this temperature. At −79° C. the per cent motile sperm in coconut milk citrate plus egg yolk diluent did not differ markedly from that of egg yolk citrate, but was conspicuously better than the motility of sperm extended in glycerol skimmilk after ten days of storage. The experimental results indicate that a combination of coconut milk and egg yolk may serve

as a satisfactory sperm extender at 5 and at −79° C.

P79. Reassessment of the "Pasteur effect" in bovine spermatozoa. R. P. SCHMIDT, J. R. LODGE, AND G. W. SALISBURY, University of Illinois, Urbana.

A study was made on the effect of isosmotic phosphate (pH 7.0) and isotonic saline diluents containing fructose on the presence or absence of a Pasteur effect by washed sperm and semen. The values (a mean of 40 observations on ten ejaculates at 37° C. during 4 hr.) for oxygen uptake, aerobic and anaerobic fructolysis, aerobic and anaerobic lactic acid production per 100×10^6 cells were as follows: washed cells in saline, 33.2 μl., −42.3 μg., −37.3 μg., +8.3 μg., +8.9 μg.; washed in phosphate, 14.4 μl., −102.8 μg., −178.5 μg., +53.6 μg., +101.8 μg.; semen in saline, 42.8 μl., +82.3 μg., −323.3 μg., −23.1 μg., +102.6 μg; semen in phosphate, 32.4 μl., +85.7 μg., −166.0 μg., +183.8 μg., and +261.1 μg., respectively. It is apparent that the Pasteur effect is dependent upon the diluent and the presence or absence of seminal plasma.

P80. Further studies on liquid nitrogen preservation of bovine semen. B. W. PICKETT, R. A. JONES, AND W. A. COWAN, University of Connecticut, Storrs, and PAUL HELLER, New England Selective Breeding Association, Inc., Woodbridge.

Semen samples from 17 bulls were diluted in an egg yolk–sodium citrate extender, split, half-stored at −79° C. (dry ice), and half-stored at −196° C. (liquid nitrogen). Spermatozoal concentration was maintained constant at 20 million progressively motile cells per milliliter prior to freezing. Per cent progressively motile cells after 1, 2, 3, 4, 6, and 8 wk. of storage were estimated, using paired samples from each storage temperature and at each week. Average values over the 8-wk. period were 20.54 and 30.34 for −79 and −196° C., respectively. The difference in favor of the colder temperature was significant at $P < 0.001$. Semen from Holstein, Guernsey, Jersey, and Angus bulls was frozen, stored in liquid nitrogen (−196° C.), and used to breed unselected, first-service cows in the field. As of December, 1958, the 60–90 day nonreturns for 508 cows were 69.09%. These results indicate that liquid nitrogen is a superior refrigerant as far as motility is concerned, when compared to dry ice, and that reasonably satisfactory fertility results can be obtained with semen stored in liquid nitrogen.

P81. Prolonged maintenance of metabolically and functionally active bovine sperm at room temperatures. C. NORMAN, C. E. JOHNSON, I. D. PORTERFIELD, AND R. S. DUNBAR, JR., West Virginia University, Morgantown.

Critical laboratory evaluation of the effect of various concentrations of coconut milk on the metabolism and life-span of bovine sperm consistently indicated that a 2.16% solution of sodium citrate dihydrate, containing 15% boiled, fresh coconut milk, could maintain an economically usable number of sperm in a metabolically active state at room temperatures for from seven to ten days. The data show that live-dead ratios, as well as quantitative and qualitative estimates of motility of sperm, taken after seven days of storage at room temperature, do not differ significantly from measurements made at zero time.

Preliminary results in a Maryland–West Virginia field trial were as follows for age of sperm (days), number of first services, and % 30–60 day nonreturns, respectively, Control diluent: 1-2, 724, and 81.9 ± 2.8; Experimental diluent: 1-7, 1,220, and 70.8 ± 2.6.

These results strongly indicate that the normal fertilizing capacity of bovine sperm suspended in coconut milk–sodium citrate extender remains unimpaired during seven days of storage at room temperatures. It should also be noted that 70.8 ± 2.6% reflects the nonreturn rate for experimental sperm extender which was largely from three to seven days old at the time of insemination.

P82. Effect of promazine and chlorpromazine on the motility and fertility of bovine semen. R. H. FOOTE, Cornell University, Ithaca, New York.

Levels of promazine (Sparine) and chlorpromazine (Thorazine)* from 0 to 2,000 γ per milliliter of extended semen were tested over a 12-day storage period at 5° C. Both drugs were toxic to spermatozoa at the highest level tested, and in no instance did promazine increase sperm survival. The average percentages of motile spermatozoa in a 20% yolk-citrate-glycine-glucose extender (CU-16 minus sulfanilamide) containing 0, 20, 100, and 200 γ of chlorpromazine per milliliter were 38, 42, 47, and 51, respectively. The 100 γ and 200 γ levels were significantly superior to the control, $P < .01$. Chlorpromazine was not beneficial in yolk extenders containing sulfanilamide, and was toxic to spermatozoa in heated skimmilk.

The fertility of bull semen stored in CU-16 extender, in which sulfanilamide was replaced with 200 γ chlorpromazine per milliliter, was compared with a 50% yolk-citrate-sulfanilamide control extender. With the experimental extender, 1,324 first services resulted in 73.9% 60- to 90-day % nonreturns versus 1,497 first services and 70.3% nonreturns for the control, $P > .05$.

* Thorazine was supplied by Smith, Kline & French for the fertility trial.

P83. The production of epididymal-like ejaculated bovine spermatozoa. C. N. GRAVES AND G. W. SALISBURY, University of Illinois, Urbana.

Semen samples were collected by means of

an artificial vagina (A.V.) into a calibrated volumetric flask containing an inhibitory diluent. The diluent in the flask was prepared immediately before collection by adding water to a mixture of $NaHCO_3$, $KHCO_3$, and $H_3C_6H_5O_7 \cdot 2H_2O$. The air phase above the diluent was then replaced with CO_2. A layer of mineral oil, added to the solution immediately after collection of the semen, kept the diluted semen saturated with CO_2. Within a short time after reaching the solution, the spermatozoa became immotile, as determined in a capillary tube under the microscope. Spermatozoa collected in this manner and stored in Illini variable temperature diluent were fertile. Following collection, other samples were centrifuged, washed in a solution identical with that used in the collection, and resuspended in saline solution, at which time they soon became motile. The metabolism of these cells was studied. The O_2 uptake values (a mean of 60 observations on 15 ejaculates during 4 hr. at 37° C.) for cells in saline and cells in saline-fructose were 17.9 and 51.8 μl. per 100×10^6 cells, respectively. These results are similar to published values on epididymal spermatozoa.

P84. Calcium and magnesium determination in bovine blood serum by versene titration. GERALD M. WARD, R. C. LINDSAY, AND C. A. VAIR, Colorado State University, Fort Collins.

Blood serum calcium was determined by dilution of serum with water, adjustment to pH 12 with NaOH, and titration with ethylene-diamine tetraacetate (versene) solution with Cal Red as indicator. Magnesium was determined by buffering to pH 10.2, boiling with sodium tungstate solution to precipitate calcium, and titration with versene solution, using erichrome black as the indicator. Either analysis can be completed in from 5 to 10 min. Calcium analyses were compared with the Clark-Collip method and no significant differences were found, by the test, between the two methods. Magnesium analyses were compared to the molybdivanadate method and in few cases the versene method gave significantly higher results than the molybdivanadate method.

P85. Blood and excretion levels of calcium and phosphorus associated with varied mineral supplements fed. K. A. KENDALL AND J. H. BYERS, University of Illinois, Urbana.

Blood serum, urinary and fecal calcium, and inorganic phosphorus levels were studied in four steers. The alfalfa hay ration fed free choice was supplemented with 100 g. calcium carbonate (CC), 130 g. dicalcium phosphate (DP), or 108 g. monosodium phosphate (MSP) administered daily in gelatin capsules. Daily blood, urine, and fecal samples for six days following a 21-day preliminary period were analyzed for calcium and inorganic phosphorus. Average daily blood serum calcium levels for the CC-, DP-, and MSP-fed animals, respectively, were 11.6, 9.4, and 10.5 mg. %, and the inorganic phosphorus levels were 5.7, 6.4, and 5.8 mg. %, respectively. Average daily urinary volumes in the same order were 4,442, 4,301, and 6,603 ml., respectively, and average daily urinary calcium concentrations were 9.77, 5.04, and 5.48 mg. %. The concentrations of urinary inorganic phosphorus, respectively, were 1.57, 16.18, and 8.89 mg. %. Fecal calcium and inorganic phosphorus values will be presented.

P86. Response of lactating cows to dextrin and sodium propionate fed in the concentrate. GEORGE E. HAWKINS, Alabama Polytechnic Institute, Auburn.

Eighteen dairy cows were used in a 28-day split plot experiment to investigate effects of concentrates and hays on physiological response. Concentrates included: (a) conventional ingredients only, (b) 29.1% dextrin, and (c) 9.7% Na propionate. Hays were: AH, alfalfa; JGH, Johnsongrass; and CBGH, Coastal Bermudagrass.

All concentrates were similar as measured by actual and 4% FCM levels of milk production. Cows on CBGH produced 8% less actual milk than those on AH or JGH, but 4% FCM was similar for all hays ($P < 0.05$). Percentage butterfat in milk from cows on CBGH was greater than that of cows on other hays, and dextrin and Na propionate reduced total butterfat production ($P < 0.05$). Mean body weights changes of all concentrate groups differed ($P < 0.05$).

Molar percentages of acetate and propionate in rumen fluid were significantly correlated ($r=0.51$ and -0.56, respectively) with amount of hay eaten. Correlations between 4% FCM production and between total butterfat production and molar percentages of acetate in rumen fluid were 0.99 and 0.96, respectively.

Dextrin and Na propionate affected body weight and butterfat prdouction, but did not affect volume of milk. Proportions of acetate in rumen fluid were reduced by dextrin.

P87. A proposed method for the analysis of forages. P. J. VAN SOEST AND L. A. MOORE, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

Work is in progress on a system of feed analysis designed to overcome some of the difficulties and inaccuracies of the Weende system. The method divides the feed into extractives, obtained with alcohol and water extraction, and residue. The components of these fractions are being analyzed in detail and compared with dry matter digestibility and digestible energy on a limited number of feeds. The extractives are being analyzed for long-chain fatty acids, organic acids, pigments, sugars, tannins, soluble polysaccharides, nitrogenous material, and total organic matter. The residue is analyzed for pectin, chlorite holocellulose, and lignin. Preliminary results indicate that the content of many constituents is related to the characteristics of the species studied, mode of prepara-

tion of the feed (grass, hay, silage, etc.), and other factors besides that of nutritive value. Amounts of long-chain fatty acids in a series of brome grasses were closely related to digestible energy.

P88. Automatic recording equipment for use in energy metabolism studies with dairy cattle. W. P. FLATT AND H. F. RIGHTER, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

The quantitative measurement of gaseous exchange in open-circuit indirect calorimetry necessitates correcting the exhaust gas from a respiration chamber for changes in temperature, barometric pressure, and relative humidity. The usual procedure has been to take periodic (hourly) readings of a mercurial barometer and the temperature of wet and dry bulb thermometers. This requires the presence of an operator 24 hr. a day. Automatic instrumentation to continuously record these variables on a strip chart recording potentiometer and to transmit the values in digital form to an automatic typewriter and IBM card-punch at periodic intervals has been developed to reduce this labor requirement. The temperature of the exhaust gas is measured by a nickel resistance bulb installed in the exhaust gas line. A sensitive electric hygrometer sensing element measures relative humidity, and a pressure transducer with a counter poise to balance atmospheric pressure is used to measure barometric pressure. From data obtained by this means, the total exhaust gas may be corrected to standard temperature and pressure (0° C. 760 mm. Hg dry gas) by means of an electronic computer.

P89. A comparison of possible primers for the determination of the gross energy of urine and high moisture materials. C. E. COPPOCK AND P. J. VAN SOEST, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

In view of the variable losses encountered upon drying urine for the purpose of obtaining the energy value, an attempt was made to determine the energy value of urine directly on the liquid product via bomb calorimetry. In a search for a suitable primer, various materials were examined including: a cellulose absorbing block, ethylene glycol, paraffin oil, methyl lactate, benzoic acid, dioxane, 10% catechol in diozane, benzoyl peroxide, ethyl cellosolve, 10% resorcinol in dioxane, sucrose, anisaldehyde, cresyl methyl ether, diethanolamine, piperidine, pyridine, ethyl acetoacetate, and various combinations of these solvents. The primers were mixed directly with urine, in primer to urine ratios of 1:1, 1:2, and 1:3, respectively. The two most promising primers appeared to be pyridine and 10% catechol in dioxane. Cupric oxide, selenium dioxide, copper salicylate, sodium bismuthate, and silver sulfate were examined for catalytic activity, but with no apparent success. Even with the most promising primers it was not possible to burn all urines. The ratio in energy between primer and urine made it very difficult to obtain precision.

P90. Effect of three different levels of TDN intake upon body weight and milk production. H. L. DALTON, W. J. MILLER, AND J. D. DONKER, University of Georgia, Athens.

A set of identical Holstein-Jersey triplets was used in the experiment with environment the same for the animals during the first 192 days. During the next 245 days, animal (2) was fed hay and grain for normal growth, according to Morrison's recommended allowances. Animal (1) was fed only hay and at a TDN level 25% below (2), and animal (3) fed at a TDN level 25% above (2). The animals received the same alfalfa hay and animals (2) and (3) received the same grain mixture. At the termination of this period, weights were 504, 579, and 614 for (1), (2), and (3), respectively. From 438 days until parturition, the animals were on fescue pasture and fed Coastal Bermuda hay ad lib. The age and weights at parturition were: (1) 789 days—565 lb.; (2) 754 days—769 lb.; (3) 764 days—835 lb. During the lactation, each animal was fed 6 lb. of grain daily and treated alike in other aspects. The production of 4% FCM for 301-day lactations was as follows: (1) 5,158 lb.; (2) 4,657 lb., and (3) 4,022 lb. End of lactation weights were 666, 885, and 907 lb. for (1), (2), and (3), respectively. The animal fed at below normal TDN intake produced more milk than the others, but failed to make satisfactory weight gains. The animal fed the higher TDN level gained the most and produced the least milk.

P91. Nutritive value of orchard grass as affected by level of nitrogen fertilization and stage of maturity. J. W. BRATZLER, E. KECK, JR., L. F. MARRIOTT, AND J. B. WASHKO, Pennsylvania Agricultural Experiment Station, University Park.

First-cutting orchard grass (S-37), replicated four times for each level of nitrogen fertilization, was harvested at three stages of maturity in which there was an eight-day interval between the harvest dates for each treatment. Levels of nitrogen fertilization used were: 50, 100, 200, 200-split, and 300 lb. per acre applied as ammonium nitrate. The freshly chopped forage was dried with the aid of supplemental heat. Complete proximate analyses of the forages showed, on the dry matter basis: crude protein contents from 9 to 28%; crude fiber contents from 24 to 34%; small but consistent declines in contents of ether extract and ash with advancing stage of maturity; and a significant increase in ash content with an increase in the level of nitrogen fertilization. All forages were evaluated nutritionally for their contents of apparent digestible dry matter, digestible crude protein, and digestible energy by feeding them to three groups of four sheep

each. Each group of sheep received forage of the same stage of maturity throughout the experiment. Significant declines in nutritive value of the forages were noted with advancing stage of maturity, with the greatest difference occurring during the last eight-day interval between harvests.

P92. Nitrogen-fertilized orchardgrass hay as compared to alfalfa for milk production. C. H. RAMAGE, E. F. OGRINZ, C. EBY, AND R. E. MATHER, Rutgers Dairy Research Farm, Sussex, New Jersey.

Twelve milking cows, eight Holsteins and four Guernseys, were used to compare the consumption, and the resultant milk production, of alfalfa hay and orchardgrass hay grown with two levels of nitrogen fertilizer (50 and 200 lb. of nitrogen per acre). Lucas' adaptation of the double-reversal design, comparing three rations, was used. The experimental periods were 5 wk., each being preceded by a 1-wk. adjustment period.

The cows were fed grain at the rate of 1 lb. for each 3 lb. of 4% FCM, and all cows received 15 lb. of grass silage daily. The protein content of the alfalfa, lower protein-, and higher protein-orchardgrass hays were 16.2, 16.2, and 21.3%, respectively. Daily hay dry matter consumption was 20.5, 19.2, and 20.8 lb. Milk production (4% FCM) was 38.6, 38.8, and 39.4 lb. daily. Body weight gains during the experiment were 0.2, 16.9, and 30.4 lb. for the alfalfa, lower protein-, and higher-protein orchardgrass hay rations. None of these differences was significant. In this trial, the orchardgrass hays were at least equal to the alfalfa hay in the production of milk and the maintenance of body weight. Calculated TDN valuees for the grass hays were somewhat higher than for alfalfa.

P93. The nutritive value of reed canarygrass hays grown under different levels of nitrogen fertilization. W. V. CHALUPA, J. L. CASON, AND B. R. BAUMGARDT, Rutgers—The State University, New Brunswick, New Jersey.

Four rations, second-cutting alfalfa and three reed canarygrass hays: (1) low—0 lb. N/A first cutting, (2) medium—100 lb. N/A second cutting, (3) high—200 lb. N/A second cutting, were fed to four dairy heifers twice daily in a total collection digestion trial (4 × 4 Latin-square). The amounts of crude protein were alfalfa—18.13%, reed canarygrass hays: low—12.72%, medium—14.86%, and high—21.64%. Ether extract (%) and gross energy (cal/g) followed trends similar to protein, whereas crude fiber (%) and NFE (%) decreased with increasing applications of nitrogen. The digestion coefficients of protein were alfalfa—78.33%, reed canarygrass hays: low—66.13%, medium—67.45%, and high—78.33%. The high level of N fertilization increased the digestion coefficients of NFE, energy, and ether extract over the low level. Crude fiber digestion coefficients decreased from 67.34 to 49.26% with increasing applications of nitrogen. The digestible energy (cal/g) and TDN of alfalfa were 2,346 and 56.02%, reed canarygrass: low—2,478 and 55.93%, medium—2,407 and 53.63%, and high—2,694 and 60.42%, respectively. Increases in digestion coefficients were significant for protein (.001), NFE (.05), and energy (.01). Differences due to treatment were significant for TDN (.05) and digestible energy (.01).

P94. Effect of supplementing dry feed on the digestibility of succulent forages. J. M. WING, University of Florida, Gainesville.

Succulent forages often result in scouring, which usually can be controlled by supplementary hay or dried citrus pulp. An experiment was designed to determine whether scouring affected digestibility of forage, and if supplementation with dry feed affected the intake of fresh forage. Digestibility of dry matter in Louisiana white clover alone was 64%. When 2.5 lb. of pangola hay or 3 lb. of dried citrus pulp were supplemented, scouring was prevented and digestibility of dry matter was 59%. Digestibility of dry matter of oats alone was 70%. With addition of 3 lb. citrus pulp, it was 73%. Slightly more of both forages was consumed by supplemented animals. Supplementation did not appear to affect digestibility of these forages, but it did control scouring.

P95. The digestibility of alfalfa hay by dairy steers as influenced by calcium and phosphorus supplements. J. H. BYERS, University of Illinois, Urbana.

Four Holstein steers weighing from 600 to 900 lb. were used in standard digestion trials to determine the influence of calcium and phosphorus mineral supplements on the digestibility of alfalfa hay. Each trial consisted of a 3-wk. preliminary period followed by a six-day collection period. Good-quality alfalfa hay was fed under a controlled ad libitum system. Trace-mineralized salt was available free choice. Mineral supplements were given daily in gelatin capsules. In Trial 1, alfalfa hay was fed alone. In Trial 2, alfalfa hay was supplemented with 100 g. of calcium carbonate and in Trial 3, alfalfa hay was supplemented with 130 g. of dicalcium phosphate. Mean dry matter consumption was 10.0, 14.9, and 13.3 lb. per head per day for Trials 1, 2, and 3, respectively. Coefficients of digestibility were dry matter, 54.4, 60.2, 54.9; energy, 52.4, 60.5, 53.5; crude protein, 64.3, 70.6, 66.5; and crude fiber, 39.7, 47.2, 45.1, for Trials 1, 2, and 3, respectively. One hundred grams of calcium carbonate significantly increased the digestibility of energy, crude protein, and crude fiber, while 130 g. of dicalcium phosphate per day showed increases but not significant increases in the same factors.

P96. Digestibility of Beltsville first-cut forages as affected by date of harvest. E. A. KANE AND L. A. MOORE, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

The applicability of the Cornell formula for determining dry matter digestibility from its linear relationship to the forage harvesting date was tested on first-cutting forages of known digestibility grown at Beltsville. The 14 forages used consisted of silages, pasture grasses, and hays. Digestibilities were determined in triplicate or quadruplicate on each forage, making a total of 45 individual digestibility determinations. The agreement found between the values calculated by the Cornell formula and the conventionally determined digestibilities was unsatisfactory, as the average error was 16%. Based on a regression equation of the dry matter digestibility (γ) of the Beltsville forages on the number of days between the forage-harvesting date and April 30 (X), the following formula was obtained: $\gamma = 74.02 - 0.393\ X$. The average difference between the calculated and known digestibilities with the Beltsville formula was found to be 2.6%. The standard deviation from regression was $\pm$ 2.1. Since the growing season arrives earlier in the South than in the North, forage vegetation will be more mature on April 30 at Beltsville, Md. than at Ithaca, N. Y. It seems apparent that a correction needs to be applied to the Cornell formula, either to the April 30 date or to the regression equation, if April 30 is used, to correct for seasonal differences due to locality.

P97. Low-fat milk production from high-level feeding of ammoniated dried beet pulp. GERALD M. WARD, GEORGE VIDACS, AND W. H. PALMER, Colorado State University, Fort Collins.

Four Holstein cows received in period (I) the regular herd ration of alfalfa hay, corn silage, and 14% grain mix; (II) corn silage was dropped, hay intake reduced to 4 lb. per day, and ammoniated dried beet pulp was offered ad libitum; (III) dehydrated alfalfa pellets replaced all the hay; (IV) the regular herd ration was again fed. The average daily milk weights and thrice-weekly butterfat percentages averaged for (I) 46.6, 3.4; (II) 48.5, 2.7; (III) 44.5, 2.3; and (IV) 41.6, 3.2. Four determinations of total solids during period (III) averaged 10.62%, during (IV), 11.53%. Beet pulp consumption reached a level of 30-35 lb. per cow and remained at about that level. At no time was there any evidence of digestive disturbances. In another trial, three cows fed increasing amounts of beet pulp up to 15–25 lb. per day with long hay and corn silage showed little change in test.

P98. Effect of pelleting on rate of concentrate consumption by dairy cows. T. H. BLOSSER AND A. O. SHAW, State College of Washington, Pullman.

Eighteen cows of the Guernsey and Jersey breeds were divided into two groups of nine each for the purpose of determining whether the rate of concentrate consumption could be increased in an elevated, walk-through milking parlor by pelleting the grain mix. After a training period, during which time all cows were fed a concentrate mix ground medium-coarse and were limited to 8 min. of eating time, one group was continued on the ground concentrate, while the second was fed the same ration in the form of 7/32-in. pellets. After an experimental period of 2 wk., the groups were reversed for an additional 2 wk. Grain consumed (in 8 min.) and milking times were recorded, as well as milk weights, and per cent butterfat was determined on milk samples taken twice during each experimental period.

Following are the averages for the 1st and 2nd wk. of the experiment: (a) Eating rate (ground grain), 0.77 and 0.76 lb/min; eating rate (pellets), 0.86 and 0.94 lb/min; (b) milking time (ground grain), 6.40 and 6.33 min.; milking time (pellets), 6.44 and 6.24 min.; (c) production rate (ground grain), 2.50 and 2.03 lb/min; production rate (pellets), 2.05 and 2.12 lb/min. The difference in fat test of the milk between treatments (ground grain vs. pellets) was small and nonsignificant.

P99. Observations regarding an unclean flavor in milk produced by feeding brome grass. C. F. FOREMAN, E. W. BIRD, F. E. NELSON, AND W. S. ROSENBERGER, Iowa State College, Ames.

About 10% of the cows pastured on brome grass developed an undesirable, unclean milk flavor toward which customer reaction is violent. The flavor may not appear in the milk as it leaves the udder, but may develop after holding one or two days. After longer grazing, the flavor is strong and persistent. It is not diminished by pasteurization, removed by "vacuumizing" or by condensation (3:1). Whey from cheese made with this milk is strongly flavored. Four susceptible cows were fed brome hay. Milk from each animal showed the flavor after holding two days at least twice during a 30-day period, and milk from two cows showed the defect uniformly after holding four days. Dilution experiments suggest that as little as 10% of the milk with the defect can cause a flavor intensity objectionable to the customer. Seven cows fed brome soilage produced milk with intense "brome-unclean" flavor after storage. Two susceptible cows grazed brome pasture for 96 days. On the second, third, and fourth days after sampling, milk from one cow showed the defect 28, 20, and 47 times, respectively, and another cow, 24, 13, and 20 times at the same intervals. The flavor component(s) has not been isolated and identified. The defect has been controlled by limited pasturing of brome, supplemental hay feeding, and removing cows from brome grass at least 4 hr. before milking.

P100. A standard for the evaluation of dairy herd management.* PHILIP L. KELLY, University of Nebraska, Lincoln.

We need other standards besides average production per cow for the evaluation of the factors of herd management which pertain only to the dairy herd. The basic factors are not only how the herd is fed, the breeding used and possible disease, but also the number of calves per cow per year, the per cent of heifers raised, and the per cent of cows culled for various reasons.

In an attempt to obtain some over-all method for the evaluation of these factors, an attempt is made to use as an index the average increase in production of average age groups from 2-yr.-olds to mature cows.

A comparison is made between herds with various levels of production. The data obtained indicate that herds with low production averages of 300 lb. or less increase less with increase in age than would be expected by calculating the mature equivalent figures based on the average production of the 2-yr.-olds. In contrast, herds averaging 500 lb. of butterfat or more showed higher increases than would be indicated by these figures.

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P101. Study of the use of plastic hose for CIP milk pipelines. M. H. ALEXANDER AND E. E. ORMISTON, University of Illinois, Urbana.

This study was designed to determine the quality of milk produced when flexible, plastic hose was used as a CIP milk pipeline for conveying the milk from the barn to the milk house. Standard bacterial plate counts were made on milk at the milk house after (1) flowing through plastic lines of 65 and 100 ft. in length when poured at the barn from the milking machines into dump stations, and (2) flowing from the milk weigh pail directly to the milk house through permanent, stainless steel lines. Both systems were cleaned and sanitized by the routine system found to be satisfactory in earlier studies at this station on permanent CIP milk pipelines. The study was continued for 12 wk., with two different kinds of dump stations and with different groups of personnel. The plastic lines and the permanent, stainless steel lines were alternated weekly as the transport system for the milk from the same group of cows. The results show that flexible, plastic hose in lengths greater than 8 ft. can be satisfactorily cleaned in place. The weekly average standard plate counts on milk from the plastic hose ranged from a low of 1,400 to a high of 6,300, and were somewhat lower than those secured on the milk from the stainless steel lines.

P102. Influence of aeration upon phosphorous constituents, fructose, and lactate in bull semen incubated at 37° C. JOAN DIXON AND M. H. EHLERS, State College of Washington, Pullman.

This experiment was performed to obtain information on metabolic processes and possible energy sources for sperm. Seventeen semen samples were extended with four parts citrate and incubated 3 hr. at 37° C.: a) in culture tubes and b) as a thin layer in Erlenmeyer flasks. Sperm concentration averaged 1.12×10^9/ml semen and motility, 67%. Fructose initially was 6.04 mg/ml of semen; fructose utilization: a) 3.63 and b) 2.20 mg/ml and lactate accumulation accounted for a) 88% and b) 77% of the fructose utilized. "Total" phosphorus was 943; "acid soluble" phosphorus, 495; "easily hydrolyzable" phosphorus, 56; "nucleic acid" phosphorus, 132; and "lipid" phosphorus, 68 μg/ml of semen. Upon incubation of the semen, easily hydrolyzable phosphorus showed small but consistent increases of a) 3 and b) 6 μg/ml. The quantity of lipid phosphorus was not altered by incubation, and the other phosphorous forms showed no consistent changes. Results indicate markedly depressed fructolysis and possible increased lactate oxidation with aeration, but do not provide any evidence for utilization of semen phospholipids by sperm with or without aeration.

P103. Effect of visible light on motility, lifespan, and respiration of bovine spermatozoa. C. NORMAN, E. GOLDBERG, C. E. JOHNSON, AND I. D. PORTERFIELD, West Virginia University, Morgantown.

Constant illumination of sperm with a 40-watt white fluorescent lamp at intensities of 300 and 1,400 foot-candles resulted in inhibition of motility and respiration, followed by death of the cells. To determine the source of the photosensitive agent, this effect was studied on sperm suspended in various media, including coconut milk–citrate, Krebs-Ringer phosphate, 0.85% saline and skimmilk, and in the presence and absence of antibioties. In the skimmilk, the sperm suspension was maintained at 5° C. All other diluents were exposed to light at a temperature of 26 ± 2° C. The deleterious effect of visible light was observable in all extenders. Further, decrease in motility of the sperm and increase in per cent dead cells were directly correlated with time and intensity of irradiation.

These results, plus the observations that the effect of visible light is enhanced in the presence of air and that catalase affords some protection against this radiation, indicate that this phenomenon may be described as a photodynamic action, except that it is presumably not brought about through the agency of a fluorescent substance. It is suggested that the radiation has a direct effect upon the spermatozoa, with the photosensitive agent being located within the cell.

P104. Studies in sulfonamide inhibition of glycolysis by bovine spermatozoa. J. R. LODGE

AND H. P. BROQUIST, University of Illinois, Urbana.

Salisbury and VanDemark (*Science*, **126**: 1118. 1957) found that under appropriate conditions high CO_2 tensions markedly inhibited glycolysis of bull semen. This effect was augmented by two structurally unrelated sulfonamides, sulfanilamide (I) and Diamox (II). These latter observations have been extended. Fructolysis experiments with semen, diluent, fructose, and test substances incubated at 37° C. in a 5% CO_2–95% N_2 atmosphere were conducted. Metabolites such as *p*-aminobenzoic acid (PABA) and folic acid, known to counteract the inhibitory effects of (I) in other biological systems, did not reverse the effect of (I) on glycolysis. Hence, (I) is probably not functioning by blocking reactions mediated by PABA or related coenzymes. Under conditions presently employed, (II), though far more potent than (I) as a carbonic anhydrase inhibitor, was no more potent than (I) in inhibiting glycolysis, implying that the action of (II) is not likely related to interference with carbonic anhydrase function. Certain compounds of similar structure, but lacking the sulfonamide moiety, did not significantly inhibit glycolysis. (I) and (II) did not inhibit glycolysis by baker's yeast. Possibly, (I) and (II) prevent the transport of fructose into the sperm cell, rather than interfere with its utilization by glycolytic enzymes.

P105. Production of carnosine by bovine spermatozoa. W. R. ANDERSON AND R. J. FLIPSE, Pennsylvania Agricultural Experiment Station, University Park.

Through the use of C^{14}-labeled histidine, the dipeptide carnosine (*β*-alanyl-histidine) has been identified as a product of histidine metabolism by washed bovine spermatozoa. Spermatozoa were centrifuged from pooled ejaculates of freshly collected bovine semen, washed in 0.9% saline, and incubated aerobically in Warburg flasks with L-histidine-2-(ring) C^{14} or D,L-histidine-*α*-C^{14}. After 2 hr. incubation at 37° C., the reaction medium was deproteinized by tipping in perchloric acid from the side-arm. The medium was neutralized, chilled, and centrifuged, and the supernate chromatographed in 2-dimensional systems. Areas of radioactivity on the chromatograms were located by autoradiography on no-screen X-ray film. Individual areas thus located were excised and eluted with water for subsequent investigation. Eluate from one such area has been cochromatographed with unlabeled L-carnosine in four solvents. Further evidence that the compound is carnosine was obtained by subjecting an aliquot of the eluate to hydrolysis with HCl. The hydrolysate was then freed of HCl and chromatographed in four different solvent systems. Both *β*-alanine and histidine were identified; only the histidine spot was radioactive. Studies are under way to determine if the carnosine $\rightleftharpoons$ anserine $\rightleftharpoons$ 1-methyl histidine pathway is operative.

P106. Oxidation of fatty acids by bovine spermatozoa. R. J. FLIPSE, Pennsylvania Agricultural Experiment Station, University Park.

Studies were made comparing the yields of $C^{14}O_2$ from washed bovine spermatozoa (pooled ejaculates) incubated aerobically with carboxy-labeled fatty acids of varying carbon chain length. The mean RCY (radiochemical yield = % of the C^{14} in the substrate which was recovered in $C^{14}O_2$/hr/10^8 spermatozoa at 37°) of three trials was as follows: acetate 35, octanoate 9, butyrate 5, myristate 4, formate 1, palmitate 1, and stearate 1. A series of trials with octanoic 1-C^{14} acid as the substrate revealed that the use of N_2 in place of air as the gas phase reduced the RCY as much as 95%. Additions of ATP caused reductions of up to 50%; neither glucose nor glycerol (0.01 *M*) exerted a marked influence on RCY. When inhibitors were incubated aerobically with spermatozoa in octanoic-1-C^{14} acid, the RCY was reduced 95% by cyanide, 83% by azide, and 71% by malonate, whereas 2,4-dinitrophenol increased RCY by nearly 50%. The data will be discussed with regard to metabolism of phospholipides and the possible metabolic role of components of semen diluents.

P107. Spermatogenesis in bulls in relation to semen production. P. T. CUPPS AND R. C. LABEN, University of California, Davis.

Using the method of Ortavant, which measures the relative frequency of the stages of the seminiferous epithelial cycle, the differences in spermatogenci activity of normal bulls and bulls with different types of lowered fertility have been measured. In those bulls producing semen with lowered concentrations of sperm, certain stages appear to require a longer period of time than normal as measured by the number of tubules at a given stage. In bulls producing semen with high concentration of sperm, there is an increased number of tubules at different stages of the epithelial cycle. Preliminary observations indicate that the method may be of value in relating the cytology of the testis with sperm production.

In addition, qualitative differences in spermiogenesis are found in bulls with some types of lowered fertility.

P108. Seasonal effects on apparent fertility of dairy bulls in the Southwest. M. A. BROWN AND R. R. HARRIS, Texas A&M College, College Station.

Analysis of breeding records of about 350 females of breeding age in the Texas A&M College, Texas Agricultural Experiment Station, dairy herd for a 3-yr. period indicated that monthly 60-to-90-day nonreturn rates ranged from 47 to 71%. High nonreturn rates of 67, 64, 69, and 71% were obtained in January, March, May, and June, respectively. Lower

rates of 53, 57, 53, and 47% were experienced in July, August, November, and December, respectively. During the same 3-yr. period, daily ambient temperatures rose above 95° F. in June, July, August, and September—an average of 12, 16, 21, and 12 days, respectively.

Poor breeding results were experienced during or immediately following months when dairy bulls were exposed to extended periods of relatively high ambient temperatures. Low nonreturn rates were obtained in November and December also, when most services were to females that had calved during the hotter months.

P109. Preliminary life-expectancy estimates for desirable bulls in artificial service. R. B. Becker and P. T. Dix Arnold, Florida Agricultural Experiment Station, Gainesville.

Improvements in methods applied to artificial breeding of cattle have increased the average tenure of desirable bulls from 1.72 yr. during 1939-1947 to 3.19 yr. during 1948-1957, an increase of 85%. These tenure periods are based on completed records of 571 and 3,270 good bulls in the respective periods. Many factors have contributed toward increased efficiency and extension of tenure of bulls in the studs. These factors, derived from research and experience, included: (a) more critical selection and thorough physical examinations of bulls prior to lease or purchase; (b) better feeding, management, and veterinary care of herd sires; (c) improved techniques for collection, evaluation, processing, and delivery of semen; (d) closer cooperation of herdsmen in timely reporting of cows to be inseminated, and (e) more skillful care and use of semen in the field. These resulted in greater efficiency in use of semen, higher conceptions, and longer tenure of bulls. The 3,270 desirable bulls removed from service during 1948 to 1957 represented more current methods and, hence, were used in tabulating expected tenures by ages. Results of this analysis will be presented in tabular form.

P110. Influence of progesterone on body temperature of spayed cows. T. R. Wrenn, Joel Bitman, and J. F. Sykes, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

A technique was developed using a resistance thermometer and a strip chart recorder for the continuous measurement of body temperature of cows. The effect of progesterone administration on the vaginal temperature of four ovariectomized dairy cows was studied. After a preliminary control period of several days, injections of progesterone elicited a marked thermogenic response in the 11 trials conducted. Quantitation of the responses was accomplished by measuring with a planimeter the areas of deviation from the control temperature on the continuous time-temperature records. These values were recorded as thermal index units. The mean thermal index values for all trials during four consecutive pretreatment days were +11.2, −12.1, +0.5, and −9.5. Similar values for four successive days following treatment were +141.7, +154.3, and +138.0. More immediate temperature elevations occurred when single doses of 250 mg. progesterone were administered intraperitoneally than when given by the subcutaneous route. The advantage of continuous temperature recording in comparison to single daily observations was demonstrated.

P111. Effect of injected progesterone on corpus luteum function in cattle. R. G. Loy, R. G. Zimbelman, and L. E. Casida, University of Wisconsin, Madison.

One group of ten open heifers was injected with 1 mg. progesterone/lb body weight on Day 1 of the estrual cycle, and another on Day 5. Corpora lutea were taken on Day 14. Control corpora were removed by supra-vaginal incision during a previous cycle. Nine pregnant heifers, injected similarly on Days 35, 42, and 49 of gestation, were slaughtered on Day 56. Ten untreated, pregnant heifers served as controls. Weights of treated and control corpora lutea differed in the Day 1 group (3.63 g. vs. 5.17 g.) but not in Day 5 or pregnant groups (3.97 g. vs. 4.34 g. and 4.85 g. vs. 5.41 g.). Proportions of functional luteal cells in treated and control corpora differed in Day 1 and Day 5 groups (36.4% vs. 85.8% and 59.7% vs. 85.1%). A large increase of connective tissue in corpora lutea of pregnant heifers precluded quantitative histological evaluation. Progesterone concentrations (*J. Biol. Chem.*, **229**: 583. 1957) of treated and control corpora lutea in γ/g differed in all groups (Day 1, 28.9 vs. 48.6; Day 5, 36.1 vs. 45.7; pregnant, 7.7 vs. 15.3). Paper chromatography of the above luteal extracts removed a small amount of a contaminating steroid.

P112. The routine determination of progestins in bovine ovaries. F. Stormshak, J. Gorski,* and R. E. Erb, State College of Washington, Pullman.

The method described by Gorski *et al.* (*J. Dairy Sci.*, **41**: 1380. 1958) for the determination of progesterone and Δ^4-pregnene-20β-ol-3-one in ovaries and corpora lutea has been modified. The earlier method involved chromatographing first in the Zaffaroni formamide–Skellysolve B system followed by rechromatographing in the Bush 95% methanol–Skellysolve B system. The present method involves only the latter. The modifications are as follows: a) After extraction the ethyl acetate-steroid-fat mixture is filtered in the cold; b) following vacuum distillation, the residue is dissolved in 10 ml. of Skellysolve B and extracted by centrifuging three times with 10-ml. volumes of 70% methanol; c) the methanol is removed under N_2 in a 45° C. water bath leaving an aqueous phase of near 10 ml., which

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is extracted with redistilled benzene and dried as described by Gorski; and d) the extract is chromatographed in the Bush system, using 3-cm. strips of Whatman No. 1 filter paper previously boiled in absolute alcohol. Recovery of added progesterone (ten trials) averaged 73 ± 3.4%. Progesterone are found principally in the corpora lutea and, thus, are very low around estrus. Progesterone increases and predominates for 10-15 days after estrus and after this time appears to decline relatively more rapidly than Δ^4-pregnene-20β-ol-3-one.

P113. Induction of lactation in the bovine; 90- versus 180-day progesterone-estrogen treatment. H. H. Olson and J. R. Davis, Southern Illinois University, Carbondale.

Recent reports indicate that daily injections of progesterone and estrogen at the ratio 1,000:1 for 180 days, followed by estrogen injections for 2 wk., induced near normal milk flow in cattle. This study was undertaken to test the repeatability of these findings and a shorter treatment period.

Two infertile nulliparous Jerseys and two infertile parous Guernseys were used in this trial. One Jersey and Guernsey received daily injections of 100 mg. progesterone and 100 γ diethylstilbestrol for 90 days, followed by 3 mg. of diethylstilbestrol daily for 3 wk. On the ninth day of estrogen treatment considerable enlargement of the udder was noted, and milking began. Peak milk production of the Jersey was 25 lb. on the 18th day of lactation and of the Guernsey was 23 lb. on the 20th day.

The other Jersey and Guernsey received similar progesterone-estrogen injections for 180 days, followed by daily injections of 3 mg. diethylstilbestrol for 3 wk. Due to the poor response, 6 mg. of diethylstilbestrol were given daily for an additional week. Milking began on the 14th day of estrogen treatment, even though the udder showed only small amounts of enlargement. Maximum milk production of the Jersey was 11 lb. on the 40th day of lactation and of the Guernsey, 23 lb. on the 65th day.

P114. Water and electrolyte concentration and distribution in cow uteri. H. W. Hawk, Joel Bitman, Helene Cecil, J. N. Wiltbank, J. Bond, and J. F. Sykes, Dairy Cattle and Beef Cattle Research Branches, USDA, Beltsville, Maryland.

Uteri of four cows in estrus and six cows in mid-cycle were compared with regard to water, Na, and K concentration and extracellular-intracellular distribution. Mean water content of endometria was 89.3% in estrous cows and 86.7% in luteal-phase ones ($P<0.01$); figures for myometria were 85.00 and 82.4%, respectively ($P<0.01$). Water distribution differed in cows of the two endocrine states. In the estrous cows, 65.5% of the endometrial water was in the extracellular phase, and in luteal-phase cows, 57.0% ($P<0.05$); in the myometrium, the figures were 44.8 and 37.5%, respectively ($P<0.01$). Total Na concentrations in estrous and luteal-phase cow endometrium were 105.4 and 91.7 mM/kg wet tissue, respectively, and in myometrium, 77.0 and 67.5 mM/kg. Na varied in extracellular but not in intracellular concentration. Endometrial K concentrations in estrous and luteal-phase cows were 37.5 and 46.3 mM/kg, respectively, and myometrial K, 63.4 and 66.5 mM/kg. K/Na ratios, which might be useful as indicators of the physiological state of the uterus, were 0.36 and 0.51, respectively, in endometria of estrous and luteal-phase cows ($P<0.01$), and 0.83 and 1.00 in the myometria ($P<0.05$).

P115. Quantitative measurements of estrogens in bovine placental cotyledons. E. L. Veenhuizen and J. Gorski,* State College of Washington, Pullman.

Estradiol-17 α estradiol-17 β, and estrone content of placental cotyledons, was determined using slaughter house specimens and samples at calving. The cotyledons (200-300 g.) were extracted three times with ethyl-acetate. The supernatant was filtered through Na_2SO_4 and evaporated in a 45° C. H_2O bath under vacuum. The residue, dissolved in 10 ml. of toluene, was extracted and centrifuged three times, using 5 ml. of NaOH each time. The lipid-free aqueous phase, neutralized with 6N H_2SO_4, was extracted with benzene. Benzene was removed by evaporation under N_2 in a 45° C. H_2O bath. The extract was chromatographed in the Zaffaroni formamide/benzene–Skellysolve B system for 11 hr. The separated estrogens were located ($FeCl_3$-$K_3Fe(CN)_6$) and eluted with 8 ml. of methanol. After evaporation to dryness, fluorescence was determined (Bates and Cohen, *Endocrinology*, **47**: 182. 1950). Recovery of added estrogens approximates 65%. At various days in gestation (estimated from crown-rump lengths), total estrogens in cotyledons averaged as follows (μg/100 g.): a)105-158, 1.42±0.12 ($n=6$); b) 170-210, 105±0.24 ($n=9$); c) 230-237, 2.41±0.32 ($n=5$); d) 256-275, 3.79±0.30 ($n=3$); and e) at calving, 14.9±2.1 ($n=7$). Estradiol-17 α appears in relatively greater quantities as gestation progresses, as compared with estradiol-17 β and estrone. Changes are most noticeable after normal calving.

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P116. Further studies on the regulation of the bovine estrous cycle by oxytocin injections. William Hansel, Cornell University, Ithaca, New York.

Previous studiees have shown that daily subcutaneous injections of 100-150 U.S.P. units of oxytocin during the first seven days of the estrous cycle inhibit the formation and function of the corpus luteum and cause heifers to re-

turn to estrus at the 9th-11th day. In the present studies, precocious estrus was induced in 11 of 13 heifers injected subcutaneously witht 100 U.S.P. units of oxytocin on Days 3-6, inclusive. The same injections given on Days 0-3, inclusive, were completely ineffective in producing precocious estrus. The 100 U.S.P. units dosage proved insufficient to produce precocious estrus in lactating cows, since only one of six cows responded to it. A dosage of 400 U.S.P. units (200 units after each milking) on Days 3-6, inclusive, produced precocious estrus in four of five lactating cows. In a further attempt to find out how oxytocin produces these effects, self-retaining rubber catheters held in place by 5 cc. inflatable balloons have been inserted into the uterus through the cervix of four heifers at the time of heat. In three of the four heifers, the catheters were expelled on the eighth day and estrus occurred on the ninth day. In the fourth heifer, the catheter was expelled on the sixth day and precocious estrus did not occur.

P117. A comparison of three methods of assay for urinary estrogenic substances. D. W. Nelson, K. O. McDougell, and E. P. Smith, Montana State College, Bozeman.

The use of chemical methods, if comparable with biossay, would save considerable time and expense and would facilitate the progress on physiological problems involving urinary estrogens. In this study, the Kober color reaction and the fluoremetric methods of assay were compared with bioassay, using the immature female mouse. Aliquots of 24-hr. urine samples were extracted and the urinary estrogens separated by paper chromotography into fractions corresponding to estrone, estradiol-17-β, and estradiol-17-α. The Kober reaction and the fluorometric methods gave higher results than did bioassay for the fraction corresponding to estrone. The correlation coefficients for the Kober reaction compared with bioassay and for the fluorometric method compared with bioassay were 0.731** and 0.719,** respectively. The Kobar method compared favorably with bioassay in the assay of the fraction corresponding to estradiol-17-β ($r = 0.926^{**}$). The correlation of the fluorometric and bioassay results was not significant ($r = 0.449$). These same comparisons for the fraction corresponding to estradiol-17-α reveal that the Kober and bioassay methods were comparable ($r = 0.664^{**}$), but that the fluorometric and bioassay results compared poorly ($r = 0.003$).

P118. Methods of managing postpartum anestrus in dairy cows. Victor Hurst, South Carolina Agricultural Experiment Station, Clemson.

Cows showing no signs of estrus by 70 days postpartum were placed in one of the four following groups: Group I, no rectal examination; Group II, rectal examination of reproductive tract at 70 days postpartum, no treatment; Group III, rectal examination of reproductive tract at 70 days postpartum and subsequent twice-daily examinations until the occurrence of estrus; Group IV, removal of the corpus luteum at 70 days postpartum. A ll cows were artificially inseminated on their first expressed estrus. Results were based upon ability of the cows to become pregnant within two services.

In group I (12 cows), 8 or 67% conceived within two services. Time from calving to first estrus ranged from 73–168 days for all 12 cows, and time from calving to conception ranged from 73–186 days. In Group II (11 cows), 10 or 91% conceived within two services. Time from calving to first estrus for the 11 cows ranged from 73–144 days, and time from calving to conception ranged from 73–156 days. In Group III (11 cows), 8 or 73% conceived within two services. Time from calving to first estrus for all cows ranged from 74–101 days, and time from calving to conception ranged from 74 to 170 days. In Group IV (11 cows), eight or 73% conceived within two services. All cows ranged from 74–143 days from calving to first estrus, and time from calving to conception ranged from 74–143 days.

P119. Post-partum regression of bovine caruncles. G. B. Marion and H. T. Gier, Kansas State College, Manhattan.

Post-partum uteri of apparently normal dairy cows were studied to ascertain if processes involved might affect future pregnancies. Immediately following parturition, caruncles consist of crumpled masses of septae and blood vessels capping an endometrial stalk. The caruncular arteries are still functional at five days postpartum, although disorganization of septal structures and accumulation leucocytes indicate necrosis. By ten days, necrosis, evidenced by mass infiltration of leucocytes, results in complete septal disorganization. By 12 days, most of the caruncular mass has sloughed away, leaving the raw surface of the stalk with protruding remnants of nonfunctional blood vessels. By 15 days, the stalk has regressed somewhat and the surface is covered with white blood corpuscles and remnants of septal cells. Sloughing is complete to the stratum compactum by 20 days and the uterine epithelium has partially re-covered the caruncle. Epithelial regeneration is complete by 25 days, and subsequent regression consists of further shrinkage of the vascular bed. Clumps of leucocytes become enclosed by the regenerating epithelium and the continued presence of these cells is often associated with high embryonic mortality in subsequent pregnancies.

EXTENSION SECTION

E1. A demonstration of the TeSa butterfat test kit. B. JACQUITH, Technical Industries, Fort Lauderdale, Florida.

The TeSa reagent test is a physio-chemical reagent test for milk, milk products, cream, ice cream mix, frozen food desserts, and chocolate milk. It can be used for raw and homogenized milk. The test is performed with a single alkaline reagent and does not require a centrifuge. Its usage and limitations will be described during the demonstration.

E2. A progress report on the TeSa reagent test for butterfat. R. G. Washburn, Ohio Agricultural Experiment Station, Wooster.

A comparison of the Babcock test and the TeSa reagent test made at the Ohio Agricultural Experiment Station indicated that there was no difference in 41% of the samples and that 95% of the samples had a difference of no more than ±0.1% unit fat. Of 127 milk samples compared, analysis by the Babcock method showed that 69.3% of the duplicate samples checked identically and 30.7% had a difference of 0.1% unit fat. The same samples analyzed by the TeSa reagent test showed 74.0% of the duplicate samples checked identically and 25.2% had a difference of 0.1% unit fat. Pennsylvania State University investigators reported that, in analyzing 96 milk samples for fat by the Babcock and TeSa reagent tests, 79.19% of the samples varied by no more than ±0.1% unit of fat. Michigan State University studies showed a tendency for the TeSa test to provide higher fat values than the Babcock test. However, 90.7% of the samples from 19 herds varied by no more than ±0.1 percentage unit of fat. Field evaluation trials being conducted in Connecticut, North Carolina, and Iowa will be included in the report.

E3. The accuracy and usefulness of central processing DHIA feed records. J. D. BURKE, Cornell University, Ithaca, New York.

The feed records on DHIA and O.S. herds calculated by Central Processing have proved to be an excellent teaching demonstration. Dairymen become more aware of the actual feed requirements of cows, the importance of careful evaluation of their forage programs, and the need to feed each cow the right amount of concentrates to supplement the roughage intake.

Concentrates indicated for individual cows, a rate of roughage feeding, and a feeding index are calculated from the feed records reported. Rates of roughage feeding range from 2 to 3 lb. and feeding indices from 100 to 130. Ninety per cent of herds fall within the normal range.

New herds tend to overestimate roughage weights and quality, to overfeed low producers, and to underfeed high producers. Exact reporting of feed weights, quality codes, and dry matter are essential if the concentrates indicated are to be valid. Abnormally high or low rates of roughage feeding are returned for verification. Errors in reporting usually clear up as a result of educational meetings, letters, and circulars. Once correct reporting has been established, many dairymen follow the feeding recommendations with excellent results.

E4. The proposed DHIA record plan. (Representative from PDCA.)

The official recognition by the various purebred dairy cattle breed associations of certain DHIA records seems probable in the near future. Electronically calculated standard DHIA records would be used in the breed production testing program under certain conditions. A proposed plan for acceptance and use by the breed associations will be presented.

E5. A nationwide dairy record-keeping campaign. R. E. BURLESON, Federal Extension Service, USDA, Washington, D. C.

Records are necessary for sound dairy farming. Yet, after more than 50 yr. of DHIA work only 10% of the cows in the United States were enrolled in a record-keeping plan in 1957. Therefore, in the annual meeting at Raleigh, N. C., in June, 1958, the Extension Section of the American Dairy Science Association recommended and approved a nationwide effort to sell dairy record-keeping, to begin in January, 1959. The Extension Committee on Organization and Policy approved the idea at its June, 1959, meeting. A committee of dairy and information people of the USDA developed a record-keeping kit containing informational aids and background materials, plus press, radio, and television releases for the campaign. States used the record-keeping kit as an aid in developing appropriate materials for county distribution. Fourteen states were supplied kit covers for inserts prepared by them. Other states preferred to have their own covers for local emphasis. Secretary Benson kicked off the campaign on January 2, with a statement to the press as well as by radio. Progress made since the beginning of the campaign will be presented from a survey of the states made prior to the A.D.S.A. meeting.

E6. Visuals — good, bad, and indifferent. HADLEY READ, JOHN BEHRENS, JACK EVERLY, University of Illinois, Urbana.

The proverb that "A Picture Is Worth a Thousand Words" is simply a way of saying that visuals usually help get across a message. The right visual, properly used, can stimulate

interest, increase understanding, and contribute to retention. Unfortunately, a poor visual or a good visual, improperly used, can create confusion and detract from the message. This session is designed to review some practical ground rules for using visuals in Extension teaching. Attention will be given to: (1) planning visual support for Extension programs, (2) picking the right visuals for the job, (3) efficient production of visuals, and (4) proper use of visuals once you have them. Simple exhibits, presentation aids, and photography will be considered.

E7. The Penn State forage testing service. R. S. ADAMS, J. W. BRATZLER, AND J. E. BAYLOR, The Pennsylvania State University, University Park.

Following 2 yr. of pilot study, a Forage Testing Service was initiated on a statewide basis. Self-addressed sample cartons can be purchased for $5 each through the county agricultural extension offices. Analyses are made according to accepted procedures by a commercial laboratory under a contract with Penn State. A forage-testing report is prepared which lists the crude protein, estimated digestible protein, crude fiber, and estimated TDN contents of the sample on a dry matter and "as fed" basis. The moisture content and a quality designation also are given for each sample.

Suggested feeding programs are developed at no additional charge for all classes of farm animals, provided that all forages fed have been sampled and all necessary information has been furnished on the proper feed program request form. Detailed information will be presented regarding procedures, benefits derived, and acceptance of this service. Forms, cartons, manuals, and the Penn State Forage Sampler will be available for inspection.

E8. Influence of plane of nutrition during early life upon the performance of dairy cows. J. T. REID, Cornell University, Ithaca, New York.

In several countries during the past 20 yr., experiments have been conducted, and some are still in progress, to determine the effect of the amount of feed provided during early life upon the performance of dariy cattle during the productive life span. In some instances the results obtained have been unexpected. For example, cows fed considerably less feed as heifers than the amount usually recommended have performed during later life at least as well as cows raised on high planes of nutrition. A level of feeding which produces very rapid growth is not economical when heifers of the larger breeds are bred at 18 mo. of age. The level of feed intake during the current lactation period has a vastly greater effect upon lactation than does that during the early growing period. Age at puberty is inversely related to the amount of feed consumed. It remains to be determined whether the combination of heavy feeding and early breeding of heifers is an economical practice. As was found in studies with fruit flies, silkworms, and rats, there is an indication that the life span of cattle raised on a low plane of nutrition might be greater than that of cattle grown at a more rapid rate. An analysis of these and other findings will be made.

E9. Field observations and data on the effect of body weight on the production of dairy heifers. R. S. ADAMS AND L. W. SPECHT, The Pennsylvania State University, University Park.

Observations suggest that inadequately grown-out replacements may be largely responsible for the low production noted in a number of problem herds. A study of 1,805 Guernsey and 7,905 Holstein heifers freshening at 24–35 mo. of age revealed that tape-weights of 32% of the Guernsey and 50% of the Holstein heifers were below those found in most growth standards. For each increment of 100 lb. in body weight, there was an increase of 20 lb. in actual butterfat production for both breeds.

Data from a county with a noticeable replacement problem indicated that growth of young calves in DHIA herds was normal. However, 2- and 3-yr.-old heifers were below standard in 74% of the herds. The average butterfat production for entire herds with poor growth was 344 lb., compared to 394 lb. for standard or above herds. It is not uncommon to find milking heifers that are 300–400 lb. undersized in some problem herds. Over-conditioning appears to be more of a problem in show herds and 4-H Club heifers than in farmer herds.

E10. Automatic dairy feeding unit. G. C. SHOVE AND K. E. HARSHBARGER, University of Illinois, Urbana.

Feed-handling problems on dairy farms too often have been attacked by the use of bigger pitch forks, haphazard installation of electric motors, or addition of a shed to the barn, with little regard to any systematic analysis of the over-all problem. Dairy farmers are using many labor-saving devices and are interested in using more, but they need help in integrating these devices into an efficient, unified system. In this new study, recent developments in automatic grinding, mixing, and delivery of concentrates will be coupled with research on metering and delivery of components for roughages, to evaluate the accuracy and dependability of complete systems. After a satisfactory system for mixing concentrates and roughages in various proportions has been developed, dairy cows will be fed automatically, with similar cows assigned to the same group. In large herds, where automation of feeding may be economically feasible, four or five groups of cows may be needed, each group

being fed a mixture suitable for its level of production. The feeding will be done automatically twice daily by mechanically mixing and conveying the mixed rations to feed bunks. The biological response to automatic feeding will be evaluated.

E11. Economic aspects of mechanization on the dairy farm. R. N. VAN ARSDALL, Farm Economics Research Division, Agricultural Research Service, USDA, Urbana, Illinois.

Specialization, large-scale operation, labor-saving methods of production, and substitution of machines for manpower came to manufacturing many decades ago. Tractors and large-capacity machinery have replaced animal power in crop production within the last 30 yr. These changes have been beneficial to all concerned. Now, circumstances appear to be ripe for livestock producers and, particularly, dairymen to accept similar changes in methods of production. Many materials-handling machines have only recently moved from the gadget stage into proved and dependable equipment. A dairyman can now buy equipment to either partially or fully mechanize all major tasks in dairying. Cost relationships favor replacing labor with mechanical equipment now, more than ever before. Prices of farm machinery and equipment have doubled since 1939, but farm wage rates are more than four times as high. Also, the supply of dependable year-round workers for livestock and dairy farms is becoming progressively tighter. Dairymen characteristically work long hours for low rates of pay. Hourly labor returns can be increased through many avenues, among them, higher prices for milk, greater production per cow, better rations, lower costs for inputs, and more cows per man. Gains will be made from a combination of all these approaches, but the recent technological breakthrough in ways of handling materials stands at the front.

E12. Cow pools. FLOYD ARNOLD, Iowa State College, Ames.

The recent development of dairy enterprises commonly referred to as "Cow Pools," has aroused much interest in contract milking. In the establishments which are currently attracting the most attention, large numbers of cows owned by many dariymen are being housed, fed, and milked on contract. No one knows what effect this will have on dairying generally. Where they operate, many adjustments will have to be made by (a) participating farmers and (b) nonparticipating farmers whose markets are changed. For the farmer who places his cows under contract, changes in cropping systems, labor needs, maintenance of soil fertility with manure, and the use of equipment and buildings will be made. For the nonparticipating farmer in the area, markets may be changed. A withdrawal of a large number of cows from a milk or cream route will create new problems. If most of the milk produced in the pool is sold as Grade A, but is from cows from which the milk formerly was used for manufacturing purposes, someone else may be squeezed out. A digest of cow pool operations in Iowa will be presented.

BOOK REVIEW

DAIRY CATTLE JUDGING AND SELECTION. W. W. Yapp. John Wiley and Sons, Inc., New York, N. Y. 324 pp. 1959.

This book emphasizes that physical form and functional ability of the dairy cow are interrelated and that dairy type is largely the sum total of these two traits. Successful application requires, (a) an intimate knowledge of anatomical parts and their relationship to producing ability, (b) sufficient training and experience to develop concepts, and (c) continued observation and study to preserve and to improve these concepts. Discussion of selection has been generally limited to that portion of the problem involving type characteristics. Most will recognize that this is, indeed, a formidable task and, as the author clearly recognizes, one that is highly subjective and fully dependent on skill in judging. He further recognizes that good cowmen will vary in their evaluation of the importance of individual physical defects but that they see the cow, as a whole, in a consistent manner. Chapters 3–13 deal with relationships between type and yield, anatomical parts, growth norms, establishment of a standard of excellence, and discussions with wisely chosen illustrations of desirable and undesirable physical characteristics. The chapters, in addition, contain historical references and utilize research wherever this is possible. Chapters 14–19 detail and illustrate the physical and morphological characteristics of the dairy breeds. The last nine chapters include prediction judging, heifer evaluation, judging and selecting dairy bulls, show ring and judging techniques, herd classification, selecting project animals, selecting and preparing animals for shows and sales, creative judging, and escaping mediocrity and standardization.

Few will agree entirely with the chapters on prediction judging and evaluating dairy heifers. However, most will disagree on different things and herein lies the stimulus for further research to seek the truth. The author, throughout the book, demonstrates through his writing that he is a scientist, student, and cowman. The author's objective to provide a textbook of value to teachers, scientists, and breeders of dairy has been partially if not wholly achieved.

R. E. ERB
State College of Washington,
Pullman

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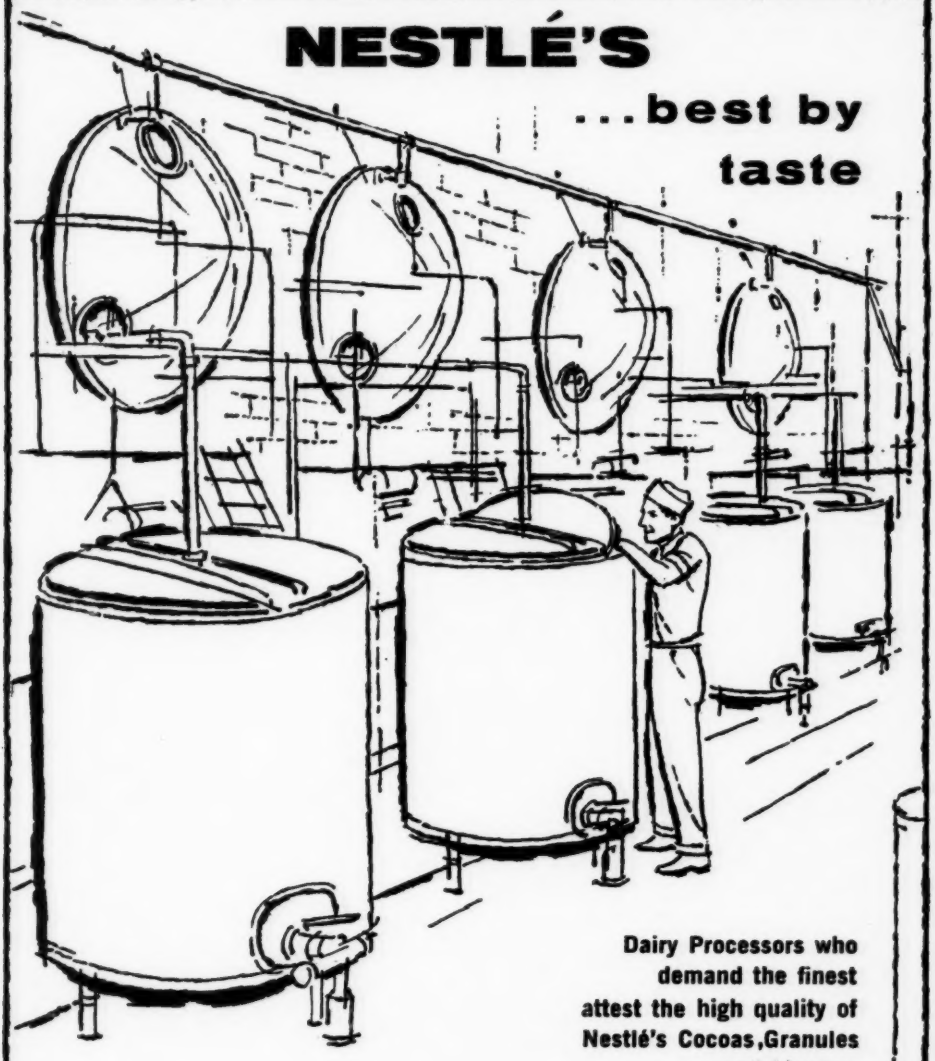

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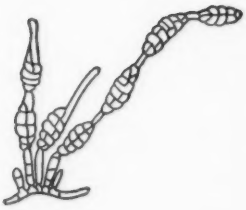

DIAGNOSTIC

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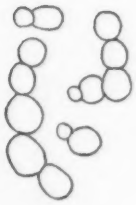

Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

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Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

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Bacto-Yeast Morphology Agar
Bacto-Yeast Carbon Base
Bacto-Yeast Nitrogen Base
Bacto-Czapek Dox Broth
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